Bescheinigung	Certificate	Attestation
Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.	The attached documents are exact copies of the European patent application described on the following page, as originally filed.	Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr.	Patent application No.	Demande de brevet n°

96870021.1 / EP96870021

The organization code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP96870021

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le President de l'Office européen des brevets
p.o.

R.C. van Dijk

Anmeldung Nr:
Application no.: 96870021.1
Demande no:

Anmeldetag:
Date of filing: 01.03.96
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

EUROSCREEN S.A.
Avenue des Bécassines 7
1160 Bruxelles/BE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

CC-chemokines receptor and nucleic acid molecule encoding said receptor

In anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen / State/Date/File no. / Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation / International Patent Classification / Classification internationale de brevets:

Am Anmeldetag benannte Vertragstaaten / Contracting states designated at date of filing / Etats contractants désignées lors du dépôt:

AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

Antrag auf Erteilung eines europäischen Patents / Request for grant of a European patent / Requête en délivrance d'un brevet européen

1

Bestätigung einer bereits durch Telekopie (Telefax) eingereichten Anmeldung / Confirmation of an application already filed by facsimile / Confirmation d'une demande déjà déposée par télécopie

☐ Ja / Yes / Oui

Wenn ja, Datum der Übermittlung der Telekopie und Name der Einreichungsbehörde / If yes, facsimile date and name of the authority with which the documents were filed / Si oui, date d'envoi de la télécopie et nom de l'autorité de dépôt

Datum / Date | Behörde / Authority / Autorité

Nur für amtlichen Gebrauch / For official use only / Cadre réservé à l'administration

Anmeldenummer / Application No. / N° de la demande	MKEY	1	96870021.1
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC	2	01-03-1996
Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))	RENA	3	
Anmeldetag / Date of filing / Date de dépôt		4	01-03-1996

Tabulatoren-Positionen / Tabulation marks / Arrêts de tabulation

Es wird die Erteilung eines europäischen Patents und gemäß Artikel 94 die Prüfung der Anmeldung beantragt / Grant of a European patent, and examination of the application under Article 94, are hereby requested / Il est demandé la délivrance d'un brevet européen et, conformément à l'article 94, l'examen de la demande — EXAM 4 — 5

☒ *Prüfungsantrag in einer zugelassenen Nichtamtssprache (siehe Merkblatt II, 5): / Request for examination in an admissible non-EPO language (see Notes II,5): / Requête en examen dans une langue non officielle autorisée (voir notice II,5).*

Verzocht wordt om onderzoek van de aanvrage als bedoeld in Art.94

Zeichen des Anmelders oder Vertreters (max. 15 Positionen) / **Applicant's or representative's reference** (maximum 15 spaces) / **Référence du demandeur ou du mandataire** (max. 15 caractères ou espaces) — AREF — 6

P.SCRE.02/EP

ANMELDER / APPLICANT / DEMANDEUR
Name / Nom — 7

EUROSCREEN S.A.

Anschrift / Address / Adresse — 8

Avenue des Becassines 7
B-1160 BRUXELLES (BELGIUM)

APPR 01 #

DEST

Zustellanschrift / Address for correspondence / Adresse pour la correspondance — 9

PADR

Staat des Wohnsitzes oder Sitzes / State of residence or of principal place of business / Etat du domicile ou du siège — 10

BELGIUM

Staatsangehörigkeit / Nationality / Nationalité — 11

BELGIAN

Telefon / Telephone / Téléphone — 12

Telex / Télex | **Telefax / Fax / Téléfax** — 13

Weitere(r) Anmelder auf Zusatzblatt / Additional applicant(s) on additional sheet / Autre(s) demandeur(s) sur feuille additionnelle — 14 ☐

VERTRETER / REPRESENTATIVE / MANDATAIRE:
Name / Nom — 15

VAN MALDEREN Joëlle

(**Nur einen** Vertreter angeben, der in das europäische Patentregister eingetragen und an den zugestellt wird / Name **only one** representative, who is to be listed in the Register of European Patents and to whom notification is to be made / N'indiquer qu'**un seul** mandataire, qui sera inscrit au Registre européen des brevets et auquel signification sera faite)

FREP 01 # # #

Geschäftsanschrift / Address of place of business / Adresse professionnelle — 16

OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
B-1083 BRUSSELS (BELGIUM)

Telefon / Telephone / Téléphone — 17

02/426 38 10

Telex / Télex | **Telefax / Fax / Téléfax** — 18

63628 PATBEL | 02/426 37 60

Weitere(r) Vertreter auf Zusatzblatt / Additional representative(s) on additional sheet / Autre(s) mandataire(s) sur feuille additionnelle — 19 ☒

TRAN | FILL

P.EURO.02/EP

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

Vollmacht / Authorisation / Pouvoir:

ist beigefügt / is enclosed / ci-joint 20 ☐

ist registriert unter Nummer / has been registered under No. / a été enregistré sous le n° GENA 21 ____ Nummer / Number / Numéro

ERFINDER / INVENTOR / INVENTEUR: INVT 20 # #

Anmelder ist (sind) alleinige(r) Erfinder / The applicant(s) is (are) the sole inventor(s) / Le(s) demandeur(s) est (sont) le (les) seul(s) inventeur(s) 22 ☐

Erfindernennung auf gesondertem Schriftstück / Designation of inventor attached / Voir la désignation de l'inventeur ci-jointe 23 X

BEZEICHNUNG DER ERFINDUNG / TITLE OF INVENTION / TITRE DE L'INVENTION: 24

[redacted]

TIDE | TIEN | TIFR

PRIORITÄTSERKLÄRUNG / DECLARATION OF PRIORITY / DECLARATION DE PRIORITE PRIO 25

Staat / State / Etat	Anmeldetag / Filing date / Date de dépôt	Aktenzeichen / Application No. / N° de la demande
01 # . . # . . . . . #		
02 # . . # . . . . . #		
03 # . . # . . . . . #		
04 # . . # . . . . . #		

Weitere Prioritätserklärung(en) auf Zusatzblatt / Additional declaration(s) of priority on additional sheet / Autre(s) déclaration(s) de priorité sur feuille additionnelle ☐

MIKROORGANISMEN

Die Erfindung betrifft einen Mikroorganismus (mehrere Mikroorganismen) oder seine (ihre) Verwendung, der (die) auf Grund des Budapester Vertrages oder eines bilateralen Abkommens zwischen der Hinterlegungsstelle und dem EPA nach Regel 28(1) a) bei einer anerkannten Hinterlegungsstelle hinterlegt worden ist (sind), um die Bedingungen für die Offenbarung der Erfindung gemäß Artikel 83 in Verbindung mit Regel 28 zu erfüllen.

MICRO-ORGANISMS

The invention relates to and/or uses (a) micro-organism(s) deposited for the purposes of disclosure pursuant to Article 83 in conjunction with Rule 28 with a depositary institution recognised within the meaning of of Rule 28(1) (a) under either the Budapest Treaty or a bilateral agreement between the institution and the EPO.

MICO 1 # | | | | | | #

26 ☐

MICRO-ORGANISMES

L'invention concerne un (plusieurs) micro-organisme(s) et/ou utilise un (plusieurs) micro-organisme(s), déposé(s) afin de satisfaire aux conditions d'exposé de l'invention prévues à l'article 83 ensemble la règle 28; à cet effet, le dépôt a été effectué auprès d'une autorité habilitée au sens de la règle 28(1) a), en vertu soit du Traité de Budapest, soit d'un accord bilatéral entre l'autorité et l'OEB.

Die Angaben nach Regel 28(1) c) sind in den technischen Anmeldungsunterlagen enthalten auf / The particulars referred to in Rule 28(1) (c) are given in the technical documents in the application on / Les indications visées à la règle 28(1) c) figurent dans les pièces techniques de la demande à la /aux 27

Seite(n) / page(s)	Zeile(n) / line(s) / ligne(s)

werden später mitgeteilt / will be submitted at a later date / seront communiquées ultérieurement 28 ☐

Die Empfangsbescheinigung(en) der Hinterlegungsstelle ist (sind) beigefügt / The receipt(s) of deposit issued by the depositary institution is (are) enclosed / Le(s) récépissé(s) de dépôt délivré(s) par l'autorité de dépôt est (sont) ci-joint(s) 29 ☐

wird (werden) nachgereicht / will be filed at a later date / sera (seront) produit(s) ultérieurement 30 ☐

EPA/EPO/OEB Form 1001.2 07.94

P.SCRE.02/EP

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

NUCLEOTID-UND AMINOSÄURESEQUENZEN / NUCLEOTIDE AND AMINO ACID SEQUENCES / SEQUENCES DE NUCLEOTIDES ET D'ACIDES AMINES SEQL (1) 31

Die Beschreibung enthält ein Sequenzprotokoll nach Regel 27a(1) / The description contains a sequence listing in accordance with Rule 27a(1) / La description contient une liste de séquences selon la règle 27bis(1) ☐

Der vorgeschriebene maschinenlesbare Datenträger ist beigefügt / The prescribed machine readable data carrier is enclosed / Le support de données prescrit déchiffrable par machine est annexé ☐

Es wird hiermit erklärt, daß die auf dem Datenträger gespeicherte Information mit dem schriftlichen Sequenzprotokoll übereinstimmt (Regel 27a(2)) / It is hereby stated that the information recorded on the data carrier is identical to the written sequence listing (Rule 27a(2)) /Il est déclaré par la présente que l'information figurant sur le support de données est identique à celle que contient la liste de séquences écrite (règle 27bis (2)) ☐

Verschiedene Anmelder für verschiedene Vertragsstaaten / Different applicants for different Contracting States / Différents demandeurs pour différents Etats contractants 32

Name(n) des (der) Anmelder(s) und benannte Vertragsstaaten / Name(s) of applicant(s) and designated Contracting States / Nom(s) du (des) demandeur(s) et des Etats contractants désignés

APPR 02 # | | | | | | | | # | | | | | | | | |

BENENNUNG VON VERTRAGSSTAATEN / DESIGNATION OF CONTRACTING STATES / DESIGNATION D'ETATS CONTRACTANTS DEST 33

Österreich / Austria / Autriche	AT	X
Belgien / Belgium / Belgique	BE	X
Schweiz und Liechtenstein / Switzerland and Liechtenstein / Suisse et Liechtenstein	CH / LI	X
Deutschland / Germany / Allemagne	DE	X
Dänemark / Denmark / Danemark	DK	X
Spanien / Spain / Espagne	ES	X
Frankreich / France / France	FR	X
Vereinigtes Königreich / United Kingdom / Royaume-Uni	GB	X
Griechenland / Greece / Grèce	GR	X
Irland / Ireland / Irlande	IE	X
Italien / Italy / Italie	IT	X
Luxemburg / Luxembourg / Luxembourg	LU	X
Monaco / Monaco / Monaco	MC	X
Niederlande / Netherlands / Pays-Bas	NL	X
Portugal / Portugal / Portugal	PT	X
Schweden / Sweden / Suède	SE	X
Finland	FI	X

(Platz für Vertragsstaaten, für die das EPÜ nach Drucklegung dieses Formblatts in Kraft tritt) (Space for Contracting States for which the EPC enters into force after this form has been printed)

(Prévu pour des Etats contractants à l'égard desquels la CBE entrera en vigueur après l'impression du présent formulaire)

VORSORGLICHE BENENNUNG SÄMTLICHER VERTRAGSSTAATEN
Die in Feld 33 angegebenen Staaten sind jene, für die die Zahlung der Benennungsgebühren vorgenommen wurde oder derzeit beabsichtigt ist. Vorsorglich werden jedoch sämtliche Staaten benannt, die zum Zeitpunkt der Einreichung dieser Anmeldung Vertragsstaaten des EPÜ sind (1.7.1994: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE). Es wird ersucht, die Benennung der hier zusätzlich benannten Vertragsstaaten als vom Anmelder zurückgenommen zu betrachten, wenn für diese Staaten die Benennungsgebühren nicht bis zum Ablauf der in Regel 85a(2) vorgesehenen Nachfrist entrichtet werden. Es wird beantragt, von der Zustellung einer Mitteilung nach Regel 85a(1) und einer Mitteilung nach Regel 69(1) betreffend die hier zusätzlich benannten Vertragsstaaten abzusehen.

PRECAUTIONARY DESIGNATION OF ALL CONTRACTING STATES
The States indicated in Section 33 are those for which it is at present intended to pay designation fees if these have not already been paid. As a precautionary measure, however, all those States which are Contracting States to the EPC at the time of filing this application are designated (1.7.1994: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE). It is hereby requested that the designation of any additional States thereby included be regarded as withdrawn by the applicant if the designation fees have not been paid by the time the period of grace allowed in Rule 85a(2) expires. It is requested that no communication under Rule 85a(1) nor any communication under Rule 69(1) concerning the additional Contracting States designated above be notified.

33a ☒

DESIGNATION A TOUTES FINS UTILES DE TOUS LES ETATS CONTRACTANTS
Les Etats indiqués à la rubrique 33 sont ceux pour lesquels le paiement des taxes de désignation a été effectué ou pour lesquels l'on se propose actuellement de payer les taxes de désignation. Toutefois, à toutes fins utiles, sont désignés tous les Etats qui sont des Etats contractants de la CBE à la date du dépôt de la demande (1.7.1994: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE). Il est demandé, au cas où les taxes de désignation pour les Etats contractants désignés à titre complémentaire ne seraient pas acquitées dans le délai supplémentaire prévu à la règle 85bis(2), que la désignation desdits Etats soit considérée comme retirée par le demandeur. Prière de ne pas procéder pour lesdits Etats contractants désignés à titre complémentaire à la signification d'une notification établie conformément à la règle 85bis(1) ou à la règle 69(1).

EPA/EPO/OEB Form 1001.3 07.94

P.SCRE.02/EP
Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

ERSTRECKUNG DES EUROPÄISCHEN PATENTS

Diese Anmeldung gilt als Antrag, die europäische Patentanmeldung und das darauf erteilte europäische Patent auf alle Nicht-Vertragsstaaten des EPÜ zu erstrecken, mit denen am Tag ihrer Einreichung „Erstreckungsabkommen" bestehen (Stand: Slowenien ab 1. März 1994). Die Erstreckung wird jedoch nur wirksam, wenn die vorgeschriebene Erstreckungsgebühr entrichtet wird.

EXTENSION OF THE EUROPEAN PATENT

This application is deemed to be a request to extend the European patent application and the European patent granted in respect of it to all non-Contracting States to the EPO with which "extension agreements" exist on the date on which the application is filed (Present situation: Slovenia from 1 March 1994). However, the extension only takes effect if the prescribed extension fee is paid.

34

EXTENSION DES EFFETS DU BREVET EUROPEEN

La présente demande est réputée constituer une requête en extension des effets de la demande de brevet européen et du brevet européen délivré sur la base de cette demande à tous les Etats non parties à la CBE avec lesquels il existe un «accord d'extension» à la date du dépôt de la demande (Situation: Slovénie à partir du 1er mars 1994). Toutefois l'extension ne produit ses effets que s'il est acquitté la taxe d'extension prescrite.

EXPT

Der Anmelder beabsichtigt derzeit, die Erstreckungsgebühr für die nachfolgend angekreuzten Staaten zu entrichten: /
The applicant currently intends to pay the extension fee for the States marked below with a cross: /
Le demandeur se propose actuellement d'acquitter la taxe d'extension pour les Etats dont le nom est coché ci-après :

Slowenien / Slovenia / Slovénie	SI	X
Lithuania	LT	X
Latvia	LV	X
Estonia	EE	X

(Platz für Staaten, mit denen nach Drucklegung dieses Formblatts „Erstreckungsabkommen" in Kraft treten) / (Space for States with which "extension agreements" enter into force after this form has been printed) / (Prévu pour des Etats à l'égard desquels des «accords d'extension» entreront en vigueur après l'impression du présent formulaire)

Die Anmeldung ist eine Teilanmeldung / The application is a divisional application / La présente demande constitue une demande divisionnaire

DFIL 9 | | | | | | #
PANR | | | | | | | | #

35 — Nummer der früheren Anmeldung / No. of earlier application / Numéro de la demande initiale

Es handelt sich um eine Anmeldung nach Art. 61(1)b) / The application is an Art. 61(1)(b) application / La présente demande constitue une demande selon l'article 61(1)b)

DFIL 9 | | | | | | #
EANR | | | | | | | | #

36 — Nummer der früheren Anmeldung / No. of earlier application / Numéro de la demande initiale

Patentansprüche / Claims / Revendications — CLMS — 37 — 76 — Zahl der Patentansprüche / Number of claims / Nombre de revendications

Weiterer Satz von Patentansprüchen (Art. 167(2)a))/ Additional set of claims (Art. 167(2)(a))/ Série supplémentaire de revendications (art. 167(2)a))

AUCL (1) — AT
AUCL (3) — ES — 38 — Zahl der Patentansprüche / Number of claims / Nombre de revendications
AUCL (4) — GR

Zur Veröffentlichung mit der Zusammenfassung wird vorgeschlagen Abbildung Nr. / With the abstract it is proposed to publish figure No. / Il est proposé de publier avec l'abrégé la figure n° — DRAW (2) — 39 — 5 — Nummer / Number / Numéro

Zusätzliche Abschrift(en) der im europäischen Recherchenbericht angeführten Schriftstücke wird (werden) beantragt / Additional copy(ies) of the documents cited in the European search report is (are) requested / Prière de fournir une (des) copie(s) supplémentaire(s) des documents cités dans le rapport de recherche européenne — ASOC — 40 — Anzahl der **zusätzlichen** Sätze von Abschriften / Number of **additional** sets of copies / Nombre de jeux **supplémentaires** de copies

EPA/EPO/OEB Form 1001.4 07.94

P_SCRE_02/EP
Raum für Zeichen des Anmelders / Space for applicant's reference / Espace reservé à la référence du demandeur

Es wird die Rückerstattung der Recherchengebühr gemäß Art. 10 GebO beantragt / Refund of the search fee is requested pursuant to Article 10 of the Rules relating to Fees / Le remboursement de la taxe de recherche est demandé en vertu de l'article 10 du règlement relatif aux taxes

41 ☐

Eine Kopie des Recherchenberichts ist beigefügt / A copy of the search report is attached / Une copie du rapport de recherche est jointe

42 ☐

AUTOMATISCHER ABBUCHUNGSAUFTRAG *(nur möglich für Inhaber von beim EPA geführten laufenden Konten)*
AUTOMATIC DEBIT ORDER *(for EPO deposit account holders only)*
ORDRE DE PRELEVEMENT AUTOMATIQUE *(uniquement possible pour les titulaires de comptes courants ouverts auprès de l'OEB)*

Das Europäische Patentamt wird hiermit beauftragt, fällig werdende Gebühren und Auslagen nach Maßgabe der Vorschriften über das automatische Abbuchungsverfahren vom nebenstehenden laufenden Konto abzubuchen / The European Patent Office is hereby authorised, under the Arrangements for the automatic debiting procedure, to debit from the deposit account opposite any fees and costs falling due / Par la présente, il est demandé à l'Office européen des brevets de prélever du compte courant ci-contre les taxes et frais venant à échéance, conformément à la réglementation relative au prélèvement automatique

DECA

Nummer des laufenden Kontos / Deposit account number / Numéro du compte courant | Name des Kontoinhabers / Account holder's name / Nom du titulaire du compte

43

Eventuelle Rückzahlungen auf das nebenstehende beim EPA geführte laufende Konto / Reimbursement, if any, to EPO deposit account opposite / Remboursements éventuels à effectuer sur le compte courant ci-contre ouvert auprès l'OEB

DEPA

Nummer des laufenden Kontos / Deposit account number / Numéro du compte courant | Name des Kontoinhabers / Account holder's name / Nom du titulaire du compte

44

Die vorgeschriebene Liste über die diesem Antrag beigefügten Unterlagen ergibt sich aus der vorbereiteten Empfangsbescheinigung (Seite 6 dieses Antrages) / The prescribed list of documents enclosed with this request is shown on the prepared receipt (page 6 of this request) / La liste prescrite des documents joints à cette requête figure sur le récépissé préétabli (page 6 de la présente requête)

45

Unterschrift(en) des (der) Anmelder(s) oder Vertreter(s) / Signature(s) of applicant(s) or representative(s) / Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

46

Für Angestellte nach Artikel 133 (3) Satz 1 mit allgemeiner Vollmacht / For employees under Article 133 (3), 1st sentence, having a general authorisation / Pour les employés mentionnés à l'article 133, paragraphe 3, 1re phrase, munis d'un pouvoir général Nr. / No. / n° :

Ort / Place / Lieu Brussels

Datum / Date March 1, 1996

VAN MALDEREn Joëlle

Name des (der) Unterzeichneten bitte mit Schreibmaschine wiederholen. Bei juristischen Personen bitte die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft mit Schreibmaschine angeben. / Please type name under signature. In case of legal persons, the position of the signatory within the company should also be typed. / Le ou les noms des signataires doivent être également dactylographiés. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires sera indiquée à la machine à écrire.

Empfangsbescheinigung / Receipt for documents / Récépissé de documents

6

(Liste der diesem Antrag beigefügten Unterlagen) (Checklist of enclosed documents) (Liste des documents annexés à la présente requête)

Es wird hiermit der Empfang der unten bezeichneten Dokumente bescheinigt / Receipt of the documents indicated below is hereby acknowledged / Nous attestons le dépôt des documents désignés ci-dessous

Wird im Falle der Einreichung der europäischen Patentanmeldung bei einer nationalen Behörde diese Empfangsbescheinigung vom Europäischen Patentamt übersandt, so ist sie als Mitteilung gemäß Regel 24(4) anzusehen (siehe Feld RENA). **Nach Erhalt der Mitteilung nach Regel 24(4) sind alle weiteren Unterlagen, die die Anmeldung betreffen, nur noch unmittelbar beim EPA einzureichen.** / If this receipt is issued by the European Patent Office and the European patent application was filed with a national authority it serves as a communication under Rule 24(4) (see Section RENA). **Once the communication under Rule 24(4) has been received, all further documents relating to the application must be sent directly to the European Patent Office.** / Si, en cas de dépôt de la demande de brevet européen auprès d'un service national, l'Office européen des brevets délivre le présent récépissé ce documents, ce récépissé est réputé être la notification visée à la règle 24(4). **Dès que la notification visée à la règle 24(4) a été reçue, tous les autres documents relatifs à la demande doivent être adressés directement à l'OEB.**

Office VAN MALDEREN
Place Reine Fabiola 6 / 1
B - 1080 BRUXELLES
Tél. 32 2 426.38.10
Fax 32 2 426.37.60

Nur für amtlichen Gebrauch / For official use only / Cadre réservé à l'administration

Datum / Date

01 -03- 1996

Unterschrift / Amtsstempel / Signature / Official stamp / Signature / Cachet officiel

Anmeldenummer / Application No. / N° de la demande		96870021.1
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC	01 -03- 1996
Zeichen des Anmelders/Vertreters / Applicant's/ Representative's ref. / Référence du demandeur ou du mandataire	AREF	P.SCRE.02/EP

Nur nach Einreichung der Anmeldung bei einer nationalen Behörde: / Only after filing of the application with a national authority: / Seulement après le dépôt de la demande auprès d'un service national:

Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))	RENA	

A. **Anmeldungsunterlagen und Prioritätsbeleg(e) / Application documents and priority document(s) / Pièces de la demande et document(s) de priorité**	47	Stückzahl / Number of copies / Nombre d'exemplaire	Blattzahl* eines Stücks / Number of sheets* in each copy / Nombre de feuilles* par exemplaire	Gesamtzahl der Abbildungen / Total number of figures / Nombre total de figures
1. Beschreibung / Description		3	30	
2. Patentansprüche / Claim(s) / Revendication(s)		3	13	
3. Ggf. unterschiedliche Patentansprüche (Art. 167(2) a)) / Any different claims (Art. 167(2)(a)) / Le cas echeant, revendications différentes (art. 167(2) a))				
4. Zeichnung(en) / Drawing(s) / Dessin(s) DRAW 1 #		3	5	
5. Zusammenfassung / Abstract / Abrégé		3	1	
6. Übersetzung der Anmeldungsunterlagen / Translation of the application documents / Traduction des pieces de la demande				
7. Prioritätsbeleg(e) / Priority document(s) / Document(s) de priorité				
8. Übersetzung des (der Prioritätsbelegs (belege) / Translation of priority document(s) / Traduction du (des) document(s) de priorité				

B. **Der Anmeldung in der eingereichten Fassung liegen folgende Unterlagen bei: / This application as filed is accompanied by the items below: / A la présente demande sont annexées les pièces suivantes:**	48	
1. Einzelvollmacht / Specific authorisation / Pouvoir particulier		
2. Allgemeine Vollmacht / General authorisation / Pouvoir général		
3. Erfindernennung / Designation of inventor / Désignation de l'inventeur		X
4. Früherer Recherchenbericht / Earlier search report / Rapport de recherche antérieure		
5. Gebührenzahlungsvordruck (EPA Form 1010) / Voucher for the settlement of fees (EPO Form 1010) / Bordereau de reglement de taxes (OEB Form 1010)		
6. Scheck *(ausgeschlossen bei Einreichung bei den nationalen Behörden)* / Cheque *(not when filing with national authorities)* / Cheque *(pas de cheque en cas de dépôt auprès des services nationaux)*		
7 Datenträger für Sequenzprotokoll / Data carrier for sequence listing / Support de données pour liste de séquences SEQL (4)		
8. Zusatzblatt / Additional sheet / Feuille additionnelle		X
9 Sonstige Unterlagen (bitte hier spezifizieren) / Other (please specify here) / Autres documents (veuillez préciser ici)		

Währung Betrag / Currency Amount / Monnaie Montant
(Ausfüllung freigestellt / optional / facultatif)

C. **Kopien dieser Empfangsbescheinigung / Copies of this receipt for documents / Copies du présent récépissé de documents**	49	3	Anzahl der Kopien / Number of copies / Nombre de copies

* Die Richtigkeit der Angabe der Blattzahl wurde bei Eingang nicht geprüft / No check was made on receipt that the number of sheets indicated was correct / L'exactitude du nombre de feuilles n'a pas été contrôlée lors du dépôt

EPA/EPO/OEB Form 1001.6 07.94

1001 6 07 94

P.SCRE.02/EP
Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

Empfangsbescheinigung / Receipt for documents / Récépissé de documents

6

(Liste der diesem Antrag beigefügten Unterlagen) (Checklist of enclosed documents) (Liste des documents annexés à la présente requête)

Es wird hiermit der Empfang der unten bezeichneten Dokumente bescheinigt / Receipt of the documents indicated below is hereby acknowledged / Nous attestons le dépôt des documents désignés ci-dessous

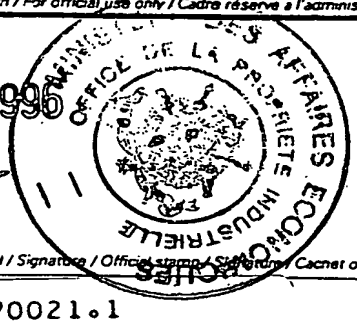
Wird im Falle der Einreichung der europäischen Patentanmeldung bei einer nationalen Behörde diese Empfangsbescheinigung vom Europäischen Patentamt übersandt, so ist sie als Mitteilung gemäß Regel 24(4) anzusehen (siehe Feld RENA). **Nach Erhalt der Mitteilung nach Regel 24(4) sind alle weiteren Unterlagen, die die Anmeldung betreffen, nur noch unmittelbar beim EPA einzureichen.** / If this receipt is issued by the European Patent Office and the European patent application was filed with a national authority it serves as a communication under Rule 24(4) (see Section RENA). **Once the communication under Rule 24(4) has been received, all further documents relating to the application must be sent directly to the European Patent Office.** / Si, en cas de dépôt de la demande de brevet européen auprès d'un service national, l'Office européen des brevets délivre le présent récépissé de documents, ce récépissé est réputé être la notification visée à la regle 24(4). **Dès que la notification visée à la règle 24(4) a été reçue, tous les autres documents relatifs à la demande doivent être adressés directement à l'OEB.**

Office VAN MALDEREN
Place Reine Fabiola 6 / 1
B - 1080 BRUXELLES
Tél. 32 2 426.38.10
Fax 32 2 426.37.60

Nur für amtlichen Gebrauch / For official use only / Cadre réservé à l'administration

Datum / Date

01 -03- 1996



Unterschrift / Amtsstempel / Signature / Official stamp / Signature / Cachet officiel

Anmeldenummer / Application No. / N° de la demande		96870021.1
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC	01 -03- 1996
Zeichen des Anmelders/Vertreters / Applicant's/ Representative's ref. / Référence du demandeur ou du mandataire	AREF	P. SCRE. 02/EP
Nur nach Einreichung der Anmeldung bei einer nationalen Behörde: / Only after filing of the application with a national authority: / Seulement apres le dépôt de la demande auprès d'un service national:		
Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))	RENA	22.03.96

			Stückzahl / Number of copies / Nombre d'exemplaire	Blattzahl* eines Stücks / Number of sheets* in each copy / Nombre de feuilles* par exemplaire	Gesamtzahl der Abbildungen / Total number of figures / Nombre total de figures
A.	**Anmeldungsunterlagen und Prioritätsbeleg(e) / Application documents and priority document(s) / Pièces de la demande et document(s) de priorité**	47			
1.	Beschreibung / Description		3	30	
2.	Patentansprüche / Claim(s) / Revendication(s)		3	13	
3.	Ggf. unterschiedliche Patentansprüche (Art. 167(2) a)) / Any different claims (Art. 167(2)(a)) / Le cas échéant, revendications différentes (art. 167(2) a))				
4.	Zeichnung(en) / Drawing(s) / Dessin(s) DRAW 1 #		3	5	
5.	Zusammenfassung / Abstract / Abrege		3	1	
6.	Übersetzung der Anmeldungsunterlagen / Translation of the application documents / Traduction des pièces de la demande				
7	Prioritatsbeleg(e) / Priority document(s) / Document(s) de priorité				
8.	Übersetzung des (der) Prioritätsbelegs (belege) / Translation of priority document(s) / Traduction du (des) document(s) de priorité				
B.	**Der Anmeldung in der eingereichten Fassung liegen folgende Unterlagen bei: / This application as filed is accompanied by the items below: / A la présente demande sont annexées les pièces suivantes:**	48			
1.	Einzelvollmacht / Specific authorisation / Pouvoir particulier				
2.	Allgemeine Vollmacht / General authorisation / Pouvoir général				
3.	Erfindernennung / Designation of inventor / Désignation de l'inventeur		X		
4	Früherer Recherchenbericht / Earlier search report / Rapport de recherche antérieure				
5.	Gebührenzahlungsvordruck (EPA Form 1010) / Voucher for the settlement of fees (EPO Form 1010) / Bordereau de reglement de taxes (OEB Form 1010)				
6	Scheck *(ausgeschlossen bei Einreichung bei den nationalen Behörden)* / Cheque *(not when filing with national authorities)* / Cheque *(pas ce cheque en cas de dépôt auprès des services nationaux)*				
7	Datenträger für Sequenzprotokoll / Data carrier for sequence listing / Support de données pour liste de sequences SEQL (4)				
8	Zusatzblatt / Additional sheet / Feuille additionnelle		X		
9	Sonstige Unterlagen (bitte hier spezifizieren) / Other (please specify here) / Autres documents (veuillez preciser ici)				
C.	**Kopien dieser Empfangsbescheinigung / Copies of this receipt for documents / Copies du présent récépissé de documents**	49	3	Anzahl der Kopien / Number of copies / Nombre de copies	

Währung Betrag / Currency Amount / Monnaie Montant
(Ausfüllung freigestellt / optional / facultatif)

* Die Richtigkeit der Angabe der Blattzahl wurde bei Eingang nicht geprüft / No check was made on receipt that the number of sheets indicated was correct / L'exactitude du nombre de feuilles n'a pas été contrôlée lors du dépôt

EPA/EPO/OEB Form 1001.6 07.94

1001 6 07 94

P. SCRE. 02/EP
Raum für Zeichen des Anmelders / Space for applicant's

ERFINDERNENNUNG / DESIGNATION OF INVENTOR / DESIGNATION DE L'INVENTEUR

(falls Anmelder nicht oder nicht allein der Erfinder ist) / (where the applicant is not the inventor or is not the sole inventor) / (si le demandeur n'est pas l'inventeur ou l'unique inventeur)

Nr. der Anmeldung oder, falls noch nicht bekannt, Bezeichnung der Erfindung
Application N° or, if not yet known, title of the invention
N° de la demande ou, si ce dernier n'est pas encore connu, titre de l'invention

CC-CHEMOKINES RECEPTOR AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR

Zeichen des Anmelders oder Vertreters
Applicant's or representative's reference
Référence du demandeur ou du mandataire
(max. 15 Positionen / max. 15 spaces / 15 caractères au maximum)

P.SCRE.02/EP

In Sachen der obenbezeichneten europäischen Patentanmeldung nennt (nennen) der (die) Unterzeichnete(n)[1]
In respect of the above European patent application I (we), the undersigned [1]
En ce qui concerne la demande de brevet européen susmentionnée le (s) soussigné(s)[1]

VAN MALDEREN Joëlle
OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
B-1083 BRUSSELS (BELGIUM)

als Erfinder[2]:
do hereby designate as inventor(s)[2]:
désigne(nt) en tant qu'inventeur(s)[2]:

SAMSON Michel
rue Victor Marquigny 11
F-94250 GENTILLY (FRANCE)

PARMENTIER Marc
Chaussée d'Uccle 304
B-1604 LINKEBEEK (BELGIUM)

VASSART Gilbert
13, Avenue Lambeau
B-1200 BRUSSELS (BELGIUM)

☐ (Weitere Erfinder sind auf einem gesonderten Blatt angegeben) / (Additional inventors indicated on supplementary sheet) / (les autres inventeurs sont mentionnés sur une feuille supplémentaire).

Der (Die) Anmelder hat (haben) das Recht auf das europäische Patent erlangt[3]
The applicant(s) has (have) acquired the right to the European patent[3]
Le(s) demandeur(s) a (ont) acquis le droit au brevet européen[3]

☐ gemäß Vertrag vom _____
under an agreement dated _____
par contrat en date du _____

☒ als Arbeitgeber
as employer(s)
en qualité d'employeur(s)

☐ durch Erbfolge
as successor(s) in title
par transfert successoral

Ort/Place/Lieu Brussels

Datum/Date March 1, 1996

Unterschrift(en) des (der) Anmelder(s) oder Vertreter(s) /
Signature(s) of applicant(s) or representative(s) /
Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

VAN MALDEREN Joëlle

Name des (der) Unterzeichneten bitte mit Schreibmaschine wiederholen. Bei juristischen Personen bitte die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft mit Schreibmaschine angeben / Please type name under signature in case of legal persons, the position of the signer within the company should also be typed / Le ou les noms des signataires doivent être également dactylographiés. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires sera indiquée à la machine à écrire

Fußnoten befinden sich auf der Rückseite / Footnotes overleaf / Le texte des renvois figure au verso

ERFINDERNENNUNG / DESIGNATION OF INVENTOR / DESIGNATION DE L'INVENTEUR

(falls Anmelder nicht oder nicht allein der Erfinder ist) / (where the applicant is not the inventor or is not the sole inventor) / (si le demandeur n'est pas l'inventeur ou l'unique inventeur)

Nr. der Anmeldung oder, falls noch nicht bekannt, Bezeichnung der Erfindung
Application N° or, if not yet known, title of the invention
N° de la demande ou, si ce dernier n'est pas encore connu, titre de l'invention

CC-CHEMOKINES RECEPTOR AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR

Zeichen des Anmelders oder Vertreters
Applicant's or representative's reference
Référence du demandeur ou du mandataire
(max. 15 Positionen / max. 15 spaces / 15 caractères au maximum)

P.SCRE.02/EP

In Sachen der obenbezeichneten europäischen Patentanmeldung nennt (nennen) der (die) Unterzeichnete(n)[1]
In respect of the above European patent application I (we), the undersigned [1]
En ce qui concerne la demande de brevet européen susmentionnée le (s) soussigné(s)[1]

VAN MALDEREN Joëlle
OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
B-1083 BRUSSELS (BELGIUM)

als Erfinder[2]:
do hereby designate as inventor(s)[2]:
désigne(nt) en tant qu'inventeur(s)[2]:

SAMSON Michel
rue Victor Marquigny 11
F-94250 GENTILLY (FRANCE)

PARMENTIER Marc
Chaussée d'Uccle 304
B-1604 LINKEBEEK (BELGIUM)

VASSART Gilbert
13, Avenue Lambeau
B-1200 BRUSSELS (BELGIUM)

☐ (Weitere Erfinder sind auf einem gesonderten Blatt angegeben) / (Additional inventors indicated on supplementary sheet) / (les autres inventeurs sont mentionnés sur une feuille supplémentaire).

Der (Die) Anmelder hat (haben) das Recht auf das europäische Patent erlangt[3]
The applicant(s) has (have) acquired the right to the European patent[3]
Le(s) demandeur(s) a (ont) acquis le droit au brevet européen[3]

☐ gemäß Vertrag vom _____
under an agreement dated _____
par contrat en date du _____

☒ als Arbeitgeber
as employer(s)
en qualité d'employeur(s)

☐ durch Erbfolge
as successor(s) in title
par transfert successoral

Ort/Place/Lieu Brussels

Datum/Date March 1, 1996

Unterschrift(en) des (der) Anmelder(s) oder Vertreter(s) /
Signature(s) of applicant(s) or representative(s) /
Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

VAN MALDEREN Joëlle

Name des (der) Unterzeichneten bitte mit Schreibmaschine wiederholen. Bei juristischen Personen bitte die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft mit Schreibmaschine angeben / Please type name under signature in case of legal persons, the position of the signer within the company should also be typed / Le ou les noms des signataires doivent être également dactylographiés. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires sera indiquée à la machine à écrire

Fußnoten befinden sich auf der Rückseite / Footnotes overleaf / Le texte des renvois figure au verso

ERFINDERNENNUNG / DESIGNATION OF INVENTOR / DESIGNATION DE L'INVENTEUR

(falls Anmelder nicht oder nicht allein der Erfinder ist) / (where the applicant is not the inventor or is not the sole inventor) / (si le demandeur n'est pas l'inventeur ou l'unique inventeur)

Nr. der Anmeldung oder, falls noch nicht bekannt, Bezeichnung der Erfindung
Application N° or, if not yet known, title of the invention
N° de la demande ou, si ce dernier n'est pas encore connu, titre de l'invention

CC-CHEMOKINES RECEPTOR AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR

Zeichen des Anmelders oder Vertreters
Applicant's or representative's reference
Référence du demandeur ou du mandataire
(max. 15 Positionen / max. 15 spaces / 15 caractères au maximum)

P.SCRE.02/EP

In Sachen der obenbezeichneten europäischen Patentanmeldung nennt (nennen) der (die) Unterzeichnete(n)[1]
In respect of the above European patent application I (we), the undersigned [1]
En ce qui concerne la demande de brevet européen susmentionnée le (s) soussigné(s)[1]

VAN MALDEREN Joëlle
OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
B-1083 BRUSSELS (BELGIUM)

als Erfinder[2]:
do hereby designate as inventor(s)[2]:
désigne(nt) en tant qu'inventeur(s)[2]:

SAMSON Michel
rue Victor Marquigny 11
F-94250 GENTILLY (FRANCE)

PARMENTIER Marc
Chaussée d'Uccle 304
B-1604 LINKEBEEK (BELGIUM)

VASSART Gilbert
13, Avenue Lambeau
B-1200 BRUSSELS (BELGIUM)

☐ (Weitere Erfinder sind auf einem gesonderten Blatt angegeben) / (Additional inventors indicated on supplementary sheet) / (les autres inventeurs sont mentionnés sur une feuille supplémentaire).

Der (Die) Anmelder hat (haben) das Recht auf das europäische Patent erlangt[3]
The applicant(s) has (have) acquired the right to the European patent[3]
Le(s) demandeur(s) a (ont) acquis le droit au brevet européen[3]

☐ gemäß Vertrag vom _____
under an agreement dated _____
par contrat en date du _____

☒ als Arbeitgeber
as employer(s)
en qualité d'employeur(s)

☐ durch Erbfolge
as successor(s) in title
par transfert successoral

Ort/Place/Lieu Brussels

Datum/Date March 1, 1996

Unterschrift(en) des (der) Anmelder(s) oder Vertreter(s) /
Signature(s) of applicant(s) or representative(s) /
Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

VAN MALDEREN Joëlle

Name des (der) Unterzeichneten bitte mit Schreibmaschine wiederholen. Bei juristischen Personen bitte die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft mit Schreibmaschine angeben / Please type name under signature in case of legal persons, the position of the signer within the company should also be typed / Le ou les noms des signataires doivent être également dactylographiés. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires sera indiquée à la machine à écrire

Fußnoten befinden sich auf der Rückseite / Footnotes overleaf / Le texte des renvois figure au verso

ERFINDERNENNUNG / DESIGNATION OF INVENTOR / DESIGNATION DE L'INVENTEUR

(falls Anmelder nicht oder nicht allein der Erfinder ist) / (where the applicant is not the inventor or is not the sole inventor) / (si le demandeur n'est pas l'inventeur ou l'unique inventeur)

Nr. der Anmeldung oder, falls noch nicht bekannt, Bezeichnung der Erfindung
Application N° or, if not yet known, title of the invention
N° de la demande ou, si ce dernier n'est pas encore connu, titre de l'invention

CC-CHEMOKINES RECEPTOR AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR

Zeichen des Anmelders oder Vertreters
Applicant's or representative's reference
Référence du demandeur ou du mandataire
(max. 15 Positionen / max. 15 spaces / 15 caractères au maximum)

P.SCRE.02/EP

In Sachen der obenbezeichneten europäischen Patentanmeldung nennt (nennen) der (die) Unterzeichnete(n)[1]
In respect of the above European patent application I (we), the undersigned [1]
En ce qui concerne la demande de brevet européen susmentionnée le (s) soussigné(s)[1]

VAN MALDEREN Joëlle
OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
B-1083 BRUSSELS (BELGIUM)

als Erfinder[2]:
do hereby designate as inventor(s)[2]:
désigne(nt) en tant qu'inventeur(s)[2]:

SAMSON Michel
rue Victor Marquigny 11
F-94250 GENTILLY (FRANCE)

PARMENTIER Marc
Chaussée d'Uccle 304
B-1604 LINKEBEEK (BELGIUM)

VASSART Gilbert
13, Avenue Lambeau
B-1200 BRUSSELS (BELGIUM)

☐ (Weitere Erfinder sind auf einem gesonderten Blatt angegeben) / (Additional inventors indicated on supplementary sheet) / (les autres inventeurs sont mentionnés sur une feuille supplémentaire).

Der (Die) Anmelder hat (haben) das Recht auf das europäische Patent erlangt[3]
The applicant(s) has (have) acquired the right to the European patent[3]
Le(s) demandeur(s) a (ont) acquis le droit au brevet européen[3]

☐ gemäß Vertrag vom _____
under an agreement dated _____
par contrat en date du _____

☒ als Arbeitgeber
as employer(s)
en qualité d'employeur(s)

☐ durch Erbfolge
as successor(s) in title
par transfert successoral

Ort/Place/Lieu Brussels

Datum/Date March 1, 1996

Unterschrift(en) des (der) Anmelder(s) oder Vertreter(s) /
Signature(s) of applicant(s) or representative(s) /
Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

VAN MALDEREN Joëlle

Name des (der) Unterzeichneten bitte mit Schreibmaschine wiederholen. Bei juristischen Personen bitte die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft mit Schreibmaschine angeben / Please type name under signature in case of legal persons, the position of the signer within the company should also be typed / Le ou les noms des signataires doivent être également dactylographiés. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires sera indiquée à la machine à écrire

Fußnoten befinden sich auf der Rückseite / Footnotes overleaf / Le texte des renvois figure au verso

bitte wenden / P.T.O. / Tour

ERFINDERNENNUNG / DESIGNATION OF INVENTOR / DESIGNATION DE L'INVENTEUR

(falls Anmelder nicht oder nicht allein der Erfinder ist) / (where the applicant is not the inventor or is not the sole inventor) / (si le demandeur n'est pas l'inventeur ou l'unique inventeur)

Nr. der Anmeldung oder, falls noch nicht bekannt, Bezeichnung der Erfindung
Application N° or, if not yet known, title of the invention
N° de la demande ou, si ce dernier n'est pas encore connu, titre de l'invention

CC-CHEMOKINES RECEPTOR AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR

Zeichen des Anmelders oder Vertreters
Applicant's or representative's reference
Référence du demandeur ou du mandataire
(max. 15 Positionen / max. 15 spaces / 15 caractères au maximum)

P.SCRE.02/EP

In Sachen der obenbezeichneten europäischen Patentanmeldung nennt (nennen) der (die) Unterzeichnete(n)[1]
In respect of the above European patent application I (we), the undersigned [1]
En ce qui concerne la demande de brevet européen susmentionnée le (s) soussigné(s)[1]

VAN MALDEREN Joëlle
OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
B-1083 BRUSSELS (BELGIUM)

als Erfinder[2]:
do hereby designate as inventor(s)[2]:
désigne(nt) en tant qu'inventeur(s)[2]:

SAMSON Michel
rue Victor Marquigny 11
F-94250 GENTILLY (FRANCE)

PARMENTIER Marc
Chaussée d'Uccle 304
B-1604 LINKEBEEK (BELGIUM)

VASSART Gilbert
13, Avenue Lambeau
B-1200 BRUSSELS (BELGIUM)

☐ (Weitere Erfinder sind auf einem gesonderten Blatt angegeben) / (Additional inventors indicated on supplementary sheet) / (les autres inventeurs sont mentionnés sur une feuille supplémentaire).

Der (Die) Anmelder hat (haben) das Recht auf das europäische Patent erlangt[3]
The applicant(s) has (have) acquired the right to the European patent[3]
Le(s) demandeur(s) a (ont) acquis le droit au brevet européen[3]

☐ gemäß Vertrag vom _____
under an agreement dated _____
par contrat en date du _____

☒ als Arbeitgeber
as employer(s)
en qualité d'employeur(s)

☐ durch Erbfolge
as successor(s) in title
par transfert successoral

Ort/Place/Lieu Brussels

Datum/Date March 1, 1996

Unterschrift(en) des (der) Anmelder(s) oder Vertreter(s) /
Signature(s) of applicant(s) or representative(s) /
Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

VAN MALDEREN Joëlle

Name des (der) Unterzeichneten bitte mit Schreibmaschine wiederholen. Bei juristischen Personen bitte die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft mit Schreibmaschine angeben / Please type name under signature in case of legal persons, the position of the signer within the company should also be typed / Le ou les noms des signataires doivent être également dactylographiés. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires sera indiquée à la machine à écrire

Fußnoten befinden sich auf der Rückseite / Footnotes overleaf / Le texte des renvois figure au verso

Antrag auf Erteilung eines europäischen Patents / Request for grant of a European patent / Requête en délivrance d'un brevet européen

Bestätigung einer bereits durch Telekopie (Telefax) eingereichten Anmeldung / Confirmation of an application already filed by facsimile / Confirmation d'une demande déjà déposée par télécopie ☐ Ja / Yes / Oui

Wenn ja, Datum der Übermittlung der Telekopie und Name der Einreichungsbehörde / If yes, facsimile date and name of the authority with which the documents were filed / Si oui, date d'envoi de la télécopie et nom de l'autorité de dépôt — Datum / Date — Behörde / Authority / Autorité

Nur für amtlichen Gebrauch / For official use only / Cadre réservé à l'administration

Anmeldenummer / Application No. / N° de la demande	MKEY	1	96870021.1
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC	2	01-03-1996
Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))	RENA	3	22.03.96
Anmeldetag / Date of filing / Date de dépôt		4	01-03-1996

Tabulatoren-Positionen / Tabulation marks / Arrêts de tabulation

Es wird die Erteilung eines europäischen Patents und gemäß Artikel 94 die Prüfung der Anmeldung beantragt / Grant of a European patent, and examination of the application under Article 94, are hereby requested / Il est demandé la délivrance d'un brevet européen et, conformément à l'article 94, l'examen de la demande EXAM 4 — 5 — ☒ *Prüfungsantrag in einer zugelassenen Nichtamtssprache (siehe Merkblatt II, 5): / Request for examination in an admissible non-EPO language (see Notes II,5): / Requête en examen dans une langue non officielle autorisée (voir notice II,5):* Verzocht wordt om onderzoek van de aanvrage als bedoeld in Art.94

Zeichen des Anmelders oder Vertreters (max. 15 Positionen) / **Applicant's or representative's reference** (maximum 15 spaces) / **Référence du demandeur ou du mandataire** (max. 15 caractères ou espaces) — AREF — 6 — P.SCRE.02/EP

ANMELDER / APPLICANT / DEMANDEUR
Name / Nom — 7 — EUROSCREEN S.A.

Anschrift / Address / Adresse — 8 — Avenue des Becassines 7
B-1160 BRUXELLES (BELGIUM)

APPR 01 # 21061581506
DEST

Zustellanschrift / Address for correspondence / Adresse pour la correspondance — 9

PADR

Staat des Wohnsitzes oder Sitzes / State of residence or of principal place of business / Etat du domicile ou du siège — 10 — BELGIUM

Staatsangehörigkeit / Nationality / Nationalité — 11 — BELGIAN

Telefon / Telephone / Téléphone — 12

Telex / Télex | **Telefax / Fax / Téléfax** — 13

Weitere(r) Anmelder auf Zusatzblatt / Additional applicant(s) on additional sheet / Autre(s) demandeur(s) sur feuille additionnelle — 14 ☐

VERTRETER / REPRESENTATIVE / MANDATAIRE:
Name / Nom — 15 — VAN MALDEREN Joëlle

(**Nur einen** Vertreter angeben, der in das europäische Patentregister eingetragen und an den zugestellt wird / Name **only one** representative, who is to be listed in the Register of European Patents and to whom notification is to be made / N'indiquer qu'**un seul** mandataire, qui sera inscrit au Registre européen des brevets et auquel signification sera faite)

FREP 01 07597121 # #1#.

Geschäftsanschrift / Address of place of business / Adresse professionnelle — 16 — OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
B-1083 BRUSSELS (BELGIUM)

Telefon / Telephone / Téléphone — 17 — 02/426 38 10

Telex / Télex | **Telefax / Fax / Téléfax** — 18 — 63628 PATBEL | 02/426 37 60

Weitere(r) Vertreter auf Zusatzblatt / Additional representative(s) on additional sheet / Autre(s) mandataire(s) sur feuille additionnelle — 19 — X

TRAN | FILL EN

P.EURO.02/EP

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

EPA/EPO/OEB Form 1001.1 07.94

1001 1 07 94

Vollmacht / Authorisation / Pouvoir:

ist beigefügt / is enclosed / ci-joint 20 ☐

ist registriert unter Nummer / has been registered under No. / a été enregistré sous le n° GENA 21 ☐ Nummer / Number / Numéro

ERFINDER / INVENTOR / INVENTEUR: INVT 20 # #

Anmelder ist (sind) alleinige(r) Erfinder / The applicant(s) is (are) the sole inventor(s) / Le(s) demandeur(s) est (sont) le (les) seul(s) inventeur(s) 22 ☐

Erfindernennung auf gesondertem Schriftstück / Designation of inventor attached / Voir la désignation de l'inventeur ci-jointe 23 ☒

BEZEICHNUNG DER ERFINDUNG / TITLE OF INVENTION / TITRE DE L'INVENTION: 24

CC-CHEMOKINES RECEPTOR AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR

TIDE | TIEN | TIFR

PRIORITÄTSERKLÄRUNG / DECLARATION OF PRIORITY / DECLARATION DE PRIORITE PRIO 25

Staat / State / Etat	Anmeldetag / Filing date / Date de dépôt	Aktenzeichen / Application No. / N° de la demande
1		
2		
3		
4		

01 # . . # . . . . . # . . . . . .

02 # . . # . . . . . # . . . . . .

03 # . . # . . . . . # . . . . . .

04 # . . # . . . . . # . . . . . .

Weitere Prioritätserklärung(en) auf Zusatzblatt / Additional declaration(s) of priority on additional sheet / Autre(s) déclaration(s) de priorité sur feuille additionnelle ☐

MIKROORGANISMEN

Die Erfindung betrifft einen Mikroorganismus (mehrere Mikroorganismen) oder seine (ihre) Verwendung, der (die) auf Grund des Budapester Vertrages oder eines bilateralen Abkommens zwischen der Hinterlegungsstelle und dem EPA nach Regel 28(1) a) bei einer anerkannten Hinterlegungsstelle hinterlegt worden ist (sind), um die Bedingungen für die Offenbarung der Erfindung gemäß Artikel 83 in Verbindung mit Regel 28 zu erfüllen.

MICRO-ORGANISMS

The invention relates to and/or uses (a) micro-organism(s) deposited for the purposes of disclosure pursuant to Article 83 in conjunction with Rule 28 with a depositary institution recognised within the meaning of of Rule 28(1) (a) under either the Budapest Treaty or a bilateral agreement between the institution and the EPO.

MICO 1 # | | | | | | #

26 ☐

MICRO-ORGANISMES

L'invention concerne un (plusieurs) micro-organisme(s) et/ou utilise un (plusieurs) micro-organisme(s), déposé(s) afin de satisfaire aux conditions d'exposé de l'invention prévues à l'article 83 ensemble la règle 28; à cet effet, le dépôt a été effectué auprès d'une autorité habilitée au sens de la règle 28(1) a), en vertu soit du Traité de Budapest, soit d'un accord bilatéral entre l'autorité et l'OEB.

Die Angaben nach Regel 28(1) c) sind in den technischen Anmeldungsunterlagen enthalten auf / The particulars referred to in Rule 28(1) (c) are given in the technical documents in the application on / Les indications visées à la règle 28(1) c) figurent dans les pièces techniques de la demande à la /aux 27

Seite(n) / page(s)	Zeile(n) / line(s) / ligne(s)

werden später mitgeteilt / will be submitted at a later date / seront communiquées ultérieurement 28 ☐

Die Empfangsbescheinigung(en) der Hinterlegungsstelle ist (sind) beigefügt / The receipt(s) of deposit issued by the depositary institution is (are) enclosed / Le(s) récépissé(s) de dépôt délivré(s) par l'autorité de dépôt est (sont) ci-joint(s) 29 ☐

wird (werden) nachgereicht / will be filed at a later date / sera (seront) produit(s) ultérieurement 30 ☐

EPA/EPO/OEB Form 1001.2 07.94

P.SCRE.02/EP

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

NUCLEOTID-UND AMINOSÄURESEQUENZEN / NUCLEOTIDE AND AMINO ACID SEQUENCES / SEQUENCES DE NUCLEOTIDES ET D'ACIDES AMINES SEQL (1) 31

Die Beschreibung enthält ein Sequenzprotokoll nach Regel 27a(1) / The description contains a sequence listing in accordance with Rule 27a(1) / La description contient une liste de séquences selon la règle 27bis(1) ☐

Der vorgeschriebene maschinenlesbare Datenträger ist beigefügt / The prescribed machine readable data carrier is enclosed / Le support de données prescrit déchiffrable par machine est annexé ☐

Es wird hiermit erklärt, daß die auf dem Datenträger gespeicherte Information mit dem schriftlichen Sequenzprotokoll übereinstimmt (Regel 27a(2)) / It is hereby stated that the information recorded on the data carrier is identical to the written sequence listing (Rule 27a(2)) /Il est déclaré par la présente que l'information figurant sur le support de données est identique à celle que contient la liste de séquences écrite (règle 27bis (2)) ☐

Verschiedene Anmelder für verschiedene Vertragsstaaten / Different applicants for different Contracting States / Différents demandeurs pour différents Etats contractants 32

Name(n) des (der) Anmelder(s) und benannte Vertragsstaaten / Name(s) of applicant(s) and designated Contracting States / Nom(s) du (des) demandeur(s) et des Etats contractants désignés

APPR 02 # | | | | | | | | # | | | | | | | | |

BENENNUNG VON VERTRAGSSTAATEN DESIGNATION OF CONTRACTING STATES DESIGNATION D'ETATS CONTRACTANTS DEST 33

Österreich / Austria / Autriche	AT	X
Belgien / Belgium / Belgique	BE	X
Schweiz und Liechtenstein / Switzerland and Liechtenstein / Suisse et Liechtenstein	CH / LI	X
Deutschland / Germany / Allemagne	DE	X
Dänemark / Denmark / Danemark	DK	X
Spanien / Spain / Espagne	ES	X
Frankreich / France / France	FR	X
Vereinigtes Königreich / United Kingdom / Royaume-Uni	GB	X
Griechenland / Greece / Grèce	GR	X
Irland / Ireland / Irlande	IE	X
Italien / Italy / Italie	IT	X
Luxemburg / Luxembourg / Luxembourg	LU	X
Monaco / Monaco / Monaco	MC	X
Niederlande / Netherlands / Pays-Bas	NL	X
Portugal / Portugal / Portugal	PT	X
Schweden / Sweden / Suède	SE	X
Finland	FI	X

(Platz für Vertragsstaaten, für die das EPÜ nach Drucklegung dieses Formblatts in Kraft tritt)

(Space for Contracting States for which the EPC enters into force after this form has been printed)

(Prévu pour des Etats contractants à l'égard desquels la CBE entrera en vigueur après l'impression du présent formulaire)

VORSORGLICHE BENENNUNG SÄMTLICHER VERTRAGSSTAATEN
Die in Feld 33 angegebenen Staaten sind jene, für die die Zahlung der Benennungsgebühren vorgenommen wurde oder derzeit beabsichtigt ist. Vorsorglich werden jedoch sämtliche Staaten benannt, die zum Zeitpunkt der Einreichung dieser Anmeldung Vertragsstaaten des EPÜ sind (1.7.1994: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE). Es wird ersucht, die Benennung der hier zusätzlich benannten Vertragsstaaten als vom Anmelder zurückgenommen zu betrachten, wenn für diese Staaten die Benennungsgebühren nicht bis zum Ablauf der in Regel 85a(2) vorgesehenen Nachfrist entrichtet werden. Es wird beantragt, von der Zustellung einer Mitteilung nach Regel 85a(1) und einer Mitteilung nach Regel 69(1) betreffend die hier zusätzlich benannten Vertragsstaaten abzusehen.

PRECAUTIONARY DESIGNATION OF ALL CONTRACTING STATES
The States indicated in Section 33 are those for which it is at present intended to pay designation fees if these have not already been paid. As a precautionary measure, however, all those States which are Contracting States to the EPC at the time of filing this application are designated (1.7.1994: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE). It is hereby requested that the designation of any additional States thereby included be regarded as withdrawn by the applicant if the designation fees have not been paid by the time the period of grace allowed in Rule 85a(2) expires. It is requested that no communication under Rule 85a(1) nor any communication under Rule 69(1) concerning the additional Contracting States designated above be notified.

33a ☒

DESIGNATION A TOUTES FINS UTILES DE TOUS LES ETATS CONTRACTANTS
Les Etats indiqués à la rubrique 33 sont ceux pour lesquels le paiement des taxes de désignation a été effectué ou pour lesquels l'on se propose actuellement de payer les taxes de désignation. Toutefois, à toutes fins utiles, sont désignés tous les Etats qui sont des Etats contractants de la CBE à la date du dépôt de la demande (1.7.1994: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE). Il est demandé, au cas où les taxes de désignation pour les Etats contractants désignés à titre complémentaire ne seraient pas acquitées dans le délai supplémentaire prévu à la règle 85bis(2), que la désignation desdits Etats soit considérée comme retirée par le demandeur. Prière de ne pas procéder pour lesdits Etats contractants désignés à titre complémentaire à la signification d'une notification établie conformément à la règle 85bis(1) ou à la règle 69(1).

ERSTRECKUNG DES EUROPÄISCHEN PATENTS

Diese Anmeldung gilt als Antrag, die europäische Patentanmeldung und das darauf erteilte europäische Patent auf alle Nicht-Vertragsstaaten des EPÜ zu erstrecken, mit denen am Tag ihrer Einreichung „Erstreckungsabkommen" bestehen (Stand: Slowenien ab 1. März 1994). Die Erstreckung wird jedoch nur wirksam, wenn die vorgeschriebene Erstreckungsgebühr entrichtet wird.

EXTENSION OF THE EUROPEAN PATENT

This application is deemed to be a request to extend the European patent application and the European patent granted in respect of it to all non-Contracting States to the EPO with which "extension agreements" exist on the date on which the application is filed (Present situation: Slovenia from 1 March 1994). However, the extension only takes effect if the prescribed extension fee is paid.

EXPT

34

EXTENSION DES EFFETS DU BREVET EUROPEEN

La présente demande est réputée constituer une requête en extension des effets de la demande de brevet européen et du brevet européen délivré sur la base de cette demande à tous les Etats non parties à la CBE avec lesquels il existe un «accord d'extension» à la date du dépôt de la demande (Situation: Slovénie à partir du 1er mars 1994). Toutefois l'extension ne produit ses effets que s'il est acquitté la taxe d'extension prescrite.

Der Anmelder beabsichtigt derzeit, die Erstreckungsgebühr für die nachfolgend angekreuzten Staaten zu entrichten: /
The applicant currently intends to pay the extension fee for the States marked below with a cross: /
Le demandeur se propose actuellement d'acquitter la taxe d'extension pour les Etats dont le nom est coché ci-après :

Slowenien / Slovenia / Slovénie	SI	x
Lithuania	LT	x
Latvia	LV	x
Estonia	EE	x

(Platz für Staaten, mit denen nach Drucklegung dieses Formblatts „Erstreckungsabkommen" in Kraft treten) / (Space for States with which "extension agreements" enter into force after this form has been printed) / (Prévu pour des Etats à l'égard desquels des «accords d'extension» entreront en vigueur après l'impression du présent formulaire)

Die Anmeldung ist eine Teilanmeldung / The application is a divisional application / La présente demande constitue une demande divisionnaire

DFIL 9 ____ #
PANR ____ #

35 ☐ Nummer der früheren Anmeldung / No. of earlier application / Numéro de la demande initiale

Es handelt sich um eine Anmeldung nach Art. 61(1)b) / The application is an Art. 61(1)(b) application / La présente demande constitue une demande selon l'article 61(1)b)

DFIL 9 ____ #
EANR ____ #

36 ☐ Nummer der früheren Anmeldung / No. of earlier application / Numéro de la demande initiale

Patentansprüche / Claims / Revendications — CLMS — 37 **76** — Zahl der Patentansprüche / Number of claims / Nombre de revendications

Weiterer Satz von Patentansprüchen (Art. 167(2)a))/ Additional set of claims (Art. 167(2)(a))/ Série supplémentaire de revendications (art. 167(2)a))

AUCL (1) — ☐ AT
AUCL (3) — 38 ☐ ES — Zahl der Patentansprüche / Number of claims / Nombre de revendications
AUCL (4) — ☐ GR

Zur Veröffentlichung mit der Zusammenfassung wird vorgeschlagen Abbildung Nr. / With the abstract it is proposed to publish figure No. / Il est proposé de publier avec l'abrégé la figure n° — DRAW (2) — 39 **5** — Nummer / Number / Numéro

Zusätzliche Abschrift(en) der im europäischen Recherchenbericht angeführten Schriftstücke wird (werden) beantragt / Additional copy(ies) of the documents cited in the European search report is (are) requested / Prière de fournir une (des) copie(s) supplémentaire(s) des documents cités dans le rapport de recherche européenne — ASOC — 40 ☐ — Anzahl der **zusätzlichen** Sätze von Abschriften / Number of **additional** sets of copies / Nombre de jeux **supplémentaires** de copies

EPA/EPO/OEB Form 1001.4 07.94

P.SCRE.02/EP
Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

Es wird die Rückerstattung der Recherchengebühr gemäß Art. 10 GebO beantragt / Refund of the search fee is requested pursuant to Article 10 of the Rules relating to Fees / Le remboursement de la taxe de recherche est demandé en vertu de l'article 10 du règlement relatif aux taxes 41 ☐

Eine Kopie des Recherchenberichts ist beigefügt / A copy of the search report is attached / Une copie du rapport de recherche est jointe 42 ☐

AUTOMATISCHER ABBUCHUNGSAUFTRAG *(nur möglich für Inhaber von beim EPA geführten laufenden Konten)*
AUTOMATIC DEBIT ORDER *(for EPO deposit account holders only)*
ORDRE DE PRELEVEMENT AUTOMATIQUE *(uniquement possible pour les titulaires de comptes courants ouverts auprès de l'OEB)*

Das Europäische Patentamt wird hiermit beauftragt, fällig werdende Gebühren und Auslagen nach Maßgabe der Vorschriften über das automatische Abbuchungsverfahren vom nebenstehenden laufenden Konto abzubuchen / The European Patent Office is hereby authorised, under the Arrangements for the automatic debiting procedure, to debit from the deposit account opposite any fees and costs falling due / Par la présente, il est demandé à l'Office européen des brevets de prélever du compte courant ci-contre les taxes et frais venant à échéance, conformément à la réglementation relative au prélèvement automatique

DECA

Nummer des laufenden Kontos / Deposit account number / Numéro du compte courant — Name des Kontoinhabers / Account holder's name / Nom du titulaire du compte

43

Eventuelle Rückzahlungen auf das nebenstehende beim EPA geführte laufende Konto / Reimbursement, if any, to EPO deposit account opposite / Remboursements éventuels à effectuer sur le compte courant ci-contre ouvert auprès l'OEB

DEPA

Nummer des laufenden Kontos / Deposit account number / Numéro du compte courant — Name des Kontoinhabers / Account holder's name / Nom du titulaire du compte

44

Die vorgeschriebene Liste über die diesem Antrag beigefügten Unterlagen ergibt sich aus der vorbereiteten Empfangsbescheinigung (Seite 6 dieses Antrages)

The prescribed list of documents enclosed with this request is shown on the prepared receipt (page 6 of this request)

45 **La liste prescrite des documents joints à cette requête figure sur le récépissé préétabli (page 6 de la présente requête)**

Unterschrift(en) des (der) Anmelder(s) oder Vertreter(s) / Signature(s) of applicant(s) or representative(s) / Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

46 **Für Angestellte nach Artikel 133 (3) Satz 1 mit allgemeiner Vollmacht / For employees under Article 133 (3), 1st sentence, having a general authorisation / Pour les employés mentionnés à l'article 133, paragraphe 3, 1re phrase, munis d'un pouvoir général Nr. / No. / n° :**

Ort / Place / Lieu Brussels

Datum / Date March 1, 1996

VAN MALDEREn Joëlle

Name des (der) Unterzeichneten bitte mit Schreibmaschine wiederholen Bei juristischen Personen bitte die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft mit Schreibmaschine angeben. / Please type name under signature. In case of legal persons, the position of the signatory within the company should also be typed. / Le ou les noms des signataires doivent être également dactylographiés. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires sera indiquée à la machine à écrire.

P.SCRE.02/EP

Raum für Zeichen des Anmelders / Space for applicant's reference / Espace réservé à la référence du demandeur

Empfangsbescheinigung / Receipt for documents / Récépissé de documents

6

(Liste der diesem Antrag beigefügten Unterlagen) (Checklist of enclosed documents) (Liste des documents annexés à la présente requête)

Es wird hiermit der Empfang der unten bezeichneten Dokumente bescheinigt / Receipt of the documents indicated below is hereby acknowledged / Nous attestons le dépôt des documents designés ci-dessous

Wird im Falle der Einreichung der europäischen Patentanmeldung bei einer nationalen Behörde diese Empfangsbescheinigung vom Europäischen Patentamt übersandt, so ist sie als Mitteilung gemäß Regel 24(4) anzusehen (siehe Feld RENA). **Nach Erhalt der Mitteilung nach Regel 24(4) sind alle weiteren Unterlagen, die die Anmeldung betreffen, nur noch unmittelbar beim EPA einzureichen.** / If this receipt is issued by the European Patent Office and the European patent application was filed with a national authority it serves as a communication under Rule 24(4) (see Section RENA). **Once the communication under Rule 24(4) has been received, all further documents relating to the application must be sent directly to the European Patent Office.** / Si, en cas de dépôt de la demande de brevet européen auprès d'un service national, l'Office européen des brevets délivre le présent récépissé de documents, ce récépissé est réputé être la notification visée à la règle 24(4). **Dès que la notification visée à la règle 24(4) a été reçue, tous les autres documents relatifs à la demande doivent être adressés directement à l'OEB.**

Office VAN MALDEREN
Place Reine Fabiola 6 / 1
B - 1080 BRUXELLES
Tél. 32 2 426.38.10
Fax 32 2 426.37.60

Nur für amtlichen Gebrauch / For official use only / Cadre réservé à l'administration

Datum / Date

- 1 -03- 1996

Unterschrift / Amtsstempel / Signature / Official stamp / Signature / Cachet officiel

Anmeldenummer / Application No. / N° de la demande			96870021.1
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC		- 1 -03- 1996
Zeichen des Anmelders/Vertreters / Applicant's/ Representative's ref. / Référence du demandeur ou du mandataire	AREF		P.SCRE.02/EP-
Nur nach Einreichung der Anmeldung bei einer nationalen Behörde: / Only after filing of the application with a national authority: / Seulement apres le dépôt de la demande auprès d'un service national:			
Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))	RENA		22.03.96

			Stückzahl / Number of copies / Nombre d'exemplaire	Blattzahl* eines Stücks / Number of sheets* in each copy / Nombre de feuilles* par exemplaire	Gesamtzahl der Abbildungen / Total number of figures / Nombre total de figures
A.	**Anmeldungsunterlagen und Prioritätsbeleg(e) / Application documents and priority document(s) / Pièces de la demande et document(s) de priorité**	47			
1.	Beschreibung / Description		3	30	
2.	Patentansprüche / Claim(s) / Revendication(s)		3	13	
3.	Ggf. unterschiedliche Patentansprüche (Art. 167(2) a)) / Any different claims (Art. 167(2)(a)) / Le cas echeant, revendications différentes (art. 167(2) a))				
4.	Zeichnung(en) / Drawing(s) / Dessin(s) DRAW 1 #		3	5	
5.	Zusammenfassung / Abstract / Abrege		3	1	
6.	Übersetzung der Anmeldungsunterlagen / Translation of the application documents / Traduction des pieces de la demande				
7.	Prioritätsbeleg(e) / Priority document(s) / Document(s) de priorité				
8.	Übersetzung des (der) Prioritätsbelegs (belege) / Translation of priority document(s) / Traduction du (des) document(s) de priorité				
B.	**Der Anmeldung in der eingereichten Fassung liegen folgende Unterlagen bei: / This application as filed is accompanied by the items below: / A la présente demande sont annexées les pièces suivantes:**	48			
1.	Einzelvollmacht / Specific authorisation / Pouvoir particulier				
2.	Allgemeine Vollmacht / General authorisation / Pouvoir général				
3.	Erfindernennung / Designation of inventor / Désignation de l'inventeur		X		
4	Früherer Recherchenbericht / Earlier search report / Rapport de recherche antérieure				
5	Gebührenzahlungsvordruck (EPA Form 1010) / Voucher for the settlement of fees (EPO Form 1010) / Bordereau de règlement de taxes (OEB Form 1010)				
6.	Scheck *(ausgeschlossen bei Einreichung bei den nationalen Behörden)* / Cheque *(not when filing with national authorities)* / Cheque *(pas de cheque en cas de dépôt auprès des services nationaux)*				
7	Datenträger für Sequenzprotokoll / Data carrier for sequence listing / Support de données pour liste de séquences SEQL (4)				
8.	Zusatzblatt / Additional sheet / Feuille additionnelle		X		
9	Sonstige Unterlagen (bitte hier spezifizieren) / Other (please specify here) / Autres documents (veuillez préciser ici)				
C.	**Kopien dieser Empfangsbescheinigung / Copies of this receipt for documents / Copies du présent récépissé de documents**	49	3	Anzahl der Kopien / Number of copies / Nombre de copies	

Währung Betrag / Currency Amount / Monnaie Montant
(Ausfüllung freigestellt / optional / facultatif)

* Die Prüfung der Angabe der Blattzahl wurde bei Eingang nicht geprüft / No check was made on receipt that the number of sheets indicated was correct / L'exactitude du nombre de feuilles n'a pas été contrôlée lors du dépôt

EPA/EPO/OEB Form 1001 6 07.94

19 ADDITIONAL REPRESENTATIVES

VAN MALDEREN Michel, VAN MALDEREN Eric
MEYERS Ernest

ERFINDERNENNUNG / DESIGNATION OF INVENTOR / DESIGNATION DE L'INVENTEUR

(falls Anmelder nicht oder nicht allein der Erfinder ist) / (where the applicant is not the inventor or is not the sole inventor) / (si le demandeur n'est pas l'inventeur ou l'unique inventeur)

Nr. der Anmeldung oder, falls noch nicht bekannt, Bezeichnung der Erfindung
Application N° or, if not yet known, title of the invention
N° de la demande ou, si ce dernier n'est pas encore connu, titre de l'invention

CC-CHEMOKINES RECEPTOR AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR

Zeichen des Anmelders oder Vertreters
Applicant's or representative's reference
Référence du demandeur ou du mandataire
(max. 15 Positionen / max. 15 spaces / 15 caractères au maximum)

P.SCRE.02/EP

In Sachen der obenbezeichneten europäischen Patentanmeldung nennt (nennen) der (die) Unterzeichnete(n)[1]
In respect of the above European patent application I (we), the undersigned [1]
En ce qui concerne la demande de brevet européen susmentionnée le (s) soussigné(s)[1]

VAN MALDEREN Joëlle
OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
B-1083 BRUSSELS (BELGIUM)

als Erfinder[2]:
do hereby designate as inventor(s)[2]:
désigne(nt) en tant qu'inventeur(s)[2]:

SAMSON Michel
rue Victor Marquigny 11
F-94250 GENTILLY (~~FRANCE~~)

PARMENTIER Marc
Chaussée d'Uccle 304
B-1604 LINKEBEEK (~~BELGIUM~~)

VASSART Gilbert
13, Avenue Lambeau
B-1200 BRUSSELS (~~BELGIUM~~)

FR / 1 / -

both BE / 1 /

☐ (Weitere Erfinder sind auf einem gesonderten Blatt angegeben) / (Additional inventors indicated on supplementary sheet) / (les autres inventeurs sont mentionnés sur une feuille supplémentaire).

Der (Die) Anmelder hat (haben) das Recht auf das europäische Patent erlangt[3]
The applicant(s) has (have) acquired the right to the European patent[3]
Le(s) demandeur(s) a (ont) acquis le droit au brevet européen[3]

☐ gemäß Vertrag vom _____
under an agreement dated _____
par contrat en date du _____

☒ als Arbeitgeber
as employer(s)
en qualité d'employeur(s)

☐ durch Erbfolge
as successor(s) in title
par transfert successoral

Ort/Place/Lieu Brussels

Datum/Date March 1, 1996

Unterschrift(en) des (der) Anmelder(s) oder Vertreter(s) /
Signature(s) of applicant(s) or representative(s) /
Signature(s) du (des) demandeur(s) ou du (des) mandataire(s)

VAN MALDEREN Joëlle

Name des (der) Unterzeichneten bitte mit Schreibmaschine wiederholen. Bei juristischen Personen bitte die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft mit Schreibmaschine angeben / Please type name under signature in case of legal persons, the position of the signer within the company should also be typed / Le ou les noms des signataires doivent être également dactylographiés. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires sera indiquée à la machine à écrire

Fußnoten befinden sich auf der Rückseite / Footnotes overleaf / Le texte des renvois figure au verso

CC-CHEMOKINES RECEPTOR AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR.

Field of the present invention.

The present invention concerns a new receptor which is stimulated by the MIP-1α, MIP-1β and RANTES chemokines.

The present invention concerns also the nucleic acid molecule encoding said receptor, the vector comprising said nucleic acid molecule, cells transformed by said vector, antibodies directed against said receptor, nucleic acid probes directed against said nucleic acid molecule, pharmaceutical compositions comprising said products and non-human transgenic animals expressing the receptor according to the invention or the nucleic acid molecule encoding said receptor.

The invention further provides a method for determining ligand binding, detecting expression, screening for drugs binding specifically to said receptor and treatments involving the receptor according to the invention.

Technological background and state of the art.

Chemotactic cytokines, or chemokines, are small signalling proteins that can be divided in two subfamilies (CC- and CXC-chemokines) depending on the relative position of the first two conserved cysteines. Interleukin 8 (IL-8) is the most studied of these proteins, but a large number of chemokines (Regulated on Activation Normal T-cell Expressed and Secreted (RANTES), Monocyte Chemoattractant Protein 1 (MCP-1), Monocyte Chemoattractant Protein 2 (MCP-2), Monocyte Chemoattractant Protein 3 (MCP-3), Growth-Related gene product α (GROα), Growth-Related gene product ß (GRO)ß, Growth-Related gene product γ (GROγ), Macrophage Inflammatory Protein 1α (MIP-1α) and ß, etc.) has now been described (Baggiolini et al., 1994). Chemokines play fundamental roles in the physiology of acute and chronic inflammatory processes as well as in the pathological dysregulations of these processes, by attracting and simulating specific subsets of leucocytes (Oppenheim et al., 1991). RANTES for example is a chemoattractant for monocytes, memory T-cells and eosinophils, and induces the release of histamine by basophils. MCP-1, released by smooth muscle cells in arteriosclerotic lesions, is considered as the factor (or one of the factors) responsible for macrophage attraction and, therefore, for the progressive aggravation of the lesions (Baggiolini et al., 1994).

MIP-1α, MIP-1ß and RANTES chemokines have recently been described as major HIV-suppressive factors produced by $CD8^+$ T-cells (Cocchi et al. (1995)). CC-chemokines are also involved in the regulation of human myeloid progenetor cell proliferation (Broxmeyer et al. (1990), Broxmeyer et al. (1993)).

Recent studies have demonstrated that the actions of CC- and CXC-chemokines are mediated by subfamilies of G protein-coupled receptors. To date, despite the numerous functions attributed to chemokines and the increasing number of biologically active ligands, only six functional receptors have been identified in human. Two receptors for interleukin-8 (IL-8) have been described [Holmes et al., 1991; Murphy & Tiffany, 1991). One (IL-8RA) binds IL-8 specifically, while the other (IL-8RB) binds IL-8 and other CXC-chemokines, like GRO. Among receptors binding CC-chemokines, a receptor, designated CC-chemokine receptor 1 (CC-CKR1), binds both RANTES and MIP-1α (Neote et al., 1993), and the CC-chemokine receptor 2 (CC-CKR2) binds MCP-1 and MCP-3 (Charo et al., 1994; Yamagami et al., 1994; Franci et al., 1995). Two additional CC-chemokine receptors were cloned recently : the CC-chemokine receptor 3 (CC-CKR3) was found to be activated by RANTES, MIP-1α and MIP-1ß (Combadiere et al., 1995) ; the CC-chemokine receptor 4 (CC-CKR4) responds to MIP-1, RANTES and MCP-1 (Power et al., 1995). In addition to these six functional receptors, a number of orphan receptors have been cloned from human and other species, that are structurally related to either CC- or CXC-chemokine receptors. These include the human BLR1 (Dobner et al., 1992), EBI1 (Birkenbach et al., 1993), LCR1 (Jazin et al., 1993), the mouse MIP-1 RL1 and MIP-1 RL2 (Gao & Murphy, 1995) and the bovine PPR1 (Matsuoka et al., 1993). Their respective ligand(s) and function(s) are unknown at present.

<u>Summary of the invention.</u>

This invention provides a new CC-chemokine receptor which is stimulated by the MIP-1ß chemokine at a concentration < 10 nM.

The stimulation of the CC-chemokine receptor according to the invention by the MIP-1ß chemokine is the biological activity (acidification rate) measured by a microphysiometer as described below.

This means that the receptor according to the invention, in presence of this agonist at this concentration or below, presents a functional response (biological activity measured by the acidification rate); while no other known CC-chemokine receptor presents said functional response when it is stimulated by said agonist at the same concentration.

Advantageously, the receptor according to the invention is also stimulated by the MIP-1α and RANTES chemokines.

In addition, said receptor is not stimulated by the MCP-1, MCP-2, MCP-3, IL-8 and GROα chemokines.

According to a preferred embodiment of the present invention, the receptor is a human receptor.

Said receptor has an amino acid sequence having more than 70% homology with the amino acid sequence shown in figure 1. Preferably, the amino acid sequence of the purified receptor according to the invention has at least the amino acid sequence shown in figure 1 or a portion thereof.

A portion of an amino sequence means a peptide or a protein having the same binding properties as the whole receptor according to the invention above-described.

The present invention is also related to a nucleic acid molecule, such as a DNA molecule or a RNA molecule, encoding the receptor according to the invention.

Preferably, said DNA molecule is a cDNA molecule or a genomic DNA molecule.

Preferably, said nucleic acid molecule has more than 70% homology to the DNA sequence shown in figure 1.

Preferably, the nucleic acid molecule according to the invention is at least the DNA sequence shown in figure 1 or a portion thereof. "A portion of a nucleic acid sequence" means a nucleic acid sequence encoding at least a portion of amino acid sequence as above-described.

The present invention is also related to a vector comprising the nucleic acid molecule according to the invention. Preferably, said vector is adapted for expression in a cell and comprises the regulatory elements necessary for expressing the amino acid molecule in said cell operatively linked to the nucleic acid sequence according to the invention as to permit expression thereof.

Preferably, said cell is chosen among the group consisting of bacterial cells, yeast cells, insect cells or mammalian cells. The vector according to the invention is a plasmid, preferably a pcDNA3 plasmid, or a virus, preferably a baculovirus, an adenovirus or a semliki forest virus.

The present invention concerns also the cell, preferably a mammalian cell, such as a CHO-K1 or a HEK293 cell, transformed by the vector according to the invention. Advantageously, said cell is non neuronal in origin and is chosen among the group consisting of CHO-K1, HEK293, BHK21, COS-7 cells.

The present invention also concerns the cell (preferably a mammalian cell such as a CHO-K1 cell) transformed by the vector according to the invention and by another vector encoding a protein enhancing the functional response in said cell. Advantageously, said protein is the Gα15 or Gα16 (G protein, α subunit).

The present invention is also related to a nucleic acid probe comprising the nucleic acid molecule according to the invention of at least 15 nucleotides capable of

specifically hybridizing with a unique sequence including with the sequence of the nucleic acid molecule encoding the receptor according to the invention. Said nucleic acid probe may be a DNA or a RNA.

The invention concerns also an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding the receptor according to the invention so as to prevent translation of said mRNA molecule or an antisense oligonucleotide having a sequence capable of specifically hybridizing to the cDNA molecule encoding the receptor according to the invention.

Said antisense oligonucleotide may comprise chemical analogs of nucleotide or substances which inactivate mRNA, or be included in an RNA molecule endowed with ribozyme activity.

Another aspect of the present invention concerns a ligand other than a chemokine (preferably an antibody) capable of binding to a receptor according to the invention and an anti-ligand (preferably also an antibody) capable of competitively inhibiting the binding of said antibody to the receptor according to the invention.

Preferably, said antibody is a monoclonal antibody.

The present invention concerns also the monoclonal antibody directed to an epitope of the receptor according to the invention and present on the surface of a cell expressing said receptor.

The invention concerns also the pharmaceutical composition comprising an effective amount of oligonucleotide according to the invention, effective to decrease the activity of said receptor by passing through a cell membrane and binding specifically with mRNA encoding the receptor according to the invention in the cell so as to prevent it

translation. The pharmaceutical composition comprises also a pharmaceutically acceptable carrier capable of passing through said cell membrane.

Preferably, in said pharmaceutical composition, the oligonucleotide is coupled to a substance, such as a ribozyme, which inactivates mRNA.

Preferably, the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cell after binding to the structure. The structure of the pharmaceutically acceptable carrier in said pharmaceutical composition is capable of binding to a receptor which is specific for a selected cell type.

Preferably, said pharmaceutical composition comprises an amount of the antibody according to the invention effective to block the binding of a ligand to the receptor according to the invention and a pharmaceutically acceptable carrier.

The present invention concerns also a transgenic non human mammal overexpressing (or expressing ectopically) the nucleic acid molecule encoding the receptor according to the invention.

The present invention also concerns a transgenic non human mammal comprising an homologous recombination knockout of the native receptor according to the invention.

According to a preferred embodiment of the invention, the transgenic non human mammal whose genome comprises antisense nucleic acid complementary to the nucleic acid according to the invention is so placed as to be transcripted into antisense mRNA which is complementary to the mRNA encoding the receptor according to the invention and which hybridizes to mRNA encoding said receptor, thereby

reducing its translation. Preferably, the transgenic non human mammal according to the invention comprises a nucleic acid molecule encoding the receptor according to the invention and comprises additionally an inducible promoter or a tissue specific regulatory element.

Preferably, the transgenic non human mammal is a mouse.

The invention relates to a method for determining whether a ligand can be specifically bound to the receptor according to the invention, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand under conditions permitting binding of ligand to such receptor and detecting the presence of any such ligand bound specifically to said receptor, thereby determining whether the ligand binds specifically to said receptor.

The invention relates to a method for determining whether a ligand can specifically bind to a receptor according to the invention, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the ligand with the membrane fraction under conditions permitting binding of the ligand to such receptor and detecting the presence of any ligand bound to said receptor, thereby determining whether the compound is capable of specifically binding to said receptor. Preferably, said method is used when the ligand is not previously known.

The invention relates to a method for determining whether a ligand is an agonist of the receptor according to the invention, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding

said receptor with the ligand under conditions permitting the activation of a functional receptor response from the cell and detecting by means of a bio-assay, such as a modification in a second messenger concentration (preferably calcium ions or inositol phosphates such as IP_3) or a modification in the cellular metabolism (preferably determined by the acidification rate of the culture medium), an increase in the receptor activity, thereby determining whether the ligand is a receptor agonist.

The invention relates to a method for determining whether a ligand is an agonist of the receptor according to the invention, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in the production of a second messenger (preferably inositol phosphates such as IP_3), an increase in the receptor activity, thereby determining whether the ligand is a receptor agonist.

The present invention relates to a method for determining whether a ligand is an antagonist of the receptor according to the invention, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in second messenger concentration (preferably calcium ions or inositol phosphates such as IP_3) or a modification in the

cellular metabolism (preferably determined by the acidification rate of the culture medium), a decrease in the receptor activity, thereby determining whether the ligand is a receptor antagonist.

The present invention relates to a method for determining whether a ligand is an antagonist of the receptor according to the invention, which comprises preparing a cell extract from cells transfected with an expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cells extract, contacting the membrane fraction with the ligand in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in the production of a second messenger, a decrease in the receptor activity, thereby determining whether the ligand is a receptor antagonist.

Preferably, the second messenger assay comprises measurement of calcium ions or inositol phosphates such as IP_3.

Preferably, the cell used in said method is a mammalian cell non neuronal in origin, such as CHO-K1, HEK293, BHK21, COS-7 cells.

The present invention also concerns the cell (preferably a mammalian cell such as a CHO-K1 cell) transformed by the vector according to the invention and by another vector encoding a protein enhancing the functional response in said cell. Advantageously, said protein is the Gα15 or Gα16 (G protein, α subunit).

In said method, the ligand is not previously known.

The invention is also related to the ligand isolated and detected by any of the preceding methods.

The present invention concerns also the pharmaceutical composition which comprises an effective amount of an agonist or an antagonist of the receptor according to the invention, effective to reduce the activity of said receptor and a pharmaceutically acceptable carrier.

For instance, said agonist or antagonist may be used in a pharmaceutical composition in the treatment of inflammatory diseases, including rheumatoid arthritis, glomerulonephritis, asthma, idiopathic pulmonary fibrosis and psoriasis, viral infections including Human Immunodeficiency Viruses 1 and 2 (HIV-1 and 2), cancer including leukaemia, atherosclerosis and/or auto-immune disorders, and the method according to the invention may be advantageously used in the detection of improved drugs which are used in the treatment of said diseases.

Therefore, the previously described methods may be used for the screening of drugs to identify drugs which specifically bind to the receptor according to the invention.

The invention is also related to the drugs isolated and detected by any of these methods.

The present invention concerns also a pharmaceutical composition comprising said drugs and a pharmaceutically acceptable carrier.

The invention is also related to a method of detecting expression of a receptor according to the invention by detecting the presence of mRNA coding for a receptor, which comprises obtaining total RNA or total mRNA from the cell and contacting the RNA or mRNA so obtained with the nucleic acid probe according to the invention under hybridizing conditions and detecting the presence of mRNA hybridized to the probe, thereby detecting the expression of the receptor by the cell.

Said hybridization conditions are stringent conditions.

The present invention concerns also the use of the pharmaceutical composition according to the invention for the treatment and/or prevention of inflammatory diseases, including rheumatoid arthritis, glomerulonephritis, asthma, idiopathic pulmonary fibrosis and psoriasis, viral infections including Human Immunodeficiency Viruses 1 and 2 (HIV-1 and 2), cancer including leukaemia, atherosclerosis and/or auto-immune disorders.

The present invention concerns also a method for diagnosing a predisposition to a disorder associated with the activity of the receptor according to the invention. Said method comprises :

a) obtaining nucleic acid molecules of subjects suffering from said disorder;

b) performing a restriction digest of said nucleic acid molecules with a panel of restriction enzymes;

c) electrophoretically separating the resulting nucleic acid fragments on a sized gel;

d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to said nucleic acid molecule and labelled with a detectable marker;

e) detecting labelled bands which have hybridized to the said nucleic acid molecule labelled with a detectable marker to create a unique band pattern specific to subjects suffering from said disorder;

f) preparing nucleic acid molecules obtained for diagnosis by step a-e; and

g) comparing the unique band pattern specific to the nucleic acid molecule of subjects suffering from the disorder from step e and the nucleic acid molecule obtained for

diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

A last aspect of the present invention concerns a method of preparing the purified receptor according to the invention, which comprises :

a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid molecules in the cell operatively linked to nucleic acid molecule encoding said receptor so as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;

b) inserting the vector of step a in a suitable host cell;

c) incubating the cell of step b under conditions allowing the expression of the receptor according to the invention;

d) recovering the receptor so obtained; and

e) purifying the receptor so recovered, thereby preparing an isolated receptor according to the invention.

Short description of the drawings.

The figure 1 represents the primary structure of a new human chemokine receptor ChemR13.

The figure 2 represents the amino acids sequence of the human chemokine receptor ChemR13 according to the invention aligned with that of the human CC-CKR1, CC-CKR2B, CC-CKR3 and CC-CKR4 receptors. Amino acids identical with the ChemR13 sequence are boxed.

The figure 3 shows the chromosomal organisation of the human CC-CKR2 and ChemR13 chemokine receptor

genes.

The figure 4 shows the functional expression of the human ChemR13 receptor in a CHO-K1 cell line.

The figure 5 represents the distribution of mRNA encoding the ChemR13 receptor in a panel of human cell lines of haematopoietic origin.

Detailed description of the invention.

1. EXPERIMENTALS

Materials

Recombinant human chemokines, including MCP-1, MIP-1α, MIP-1ß, RANTES, IL-8 and GROα were obtained from R & D Systems (London, UK). MCP-2 and MCP-3 were a gift of J. Van Damme, University of Leuven, Belgium. [^{125}I]MIP-1α (specific activity, 2200 Ci/mmol) was obtained from Dupont NEN (Brussels, Belgium). Chemokines obtained from R & D Systems were reported by the supplier as >97 % pure on SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and biologically active on a bioassay specific for each ligand. The lyophilized chemokines were dissolved as a 100 µg/ml solution in a sterile phosphate-buffered saline (PBS) and this stock solution was stored at -20° C in aliquots. Chemokines were diluted to the working concentration immediately before use. All cell lines used in the present study were obtained from the ATCC (Rockville, MD, USA).

Cloning and sequencing

The mouse MOP020 clone was obtained by low stringency polymerase chain reaction, as described previously (Libert et al., 1989; Parmentier et al., 1989), using genomic DNA as template. A human genomic DNA library (Stratagene, La Jolla, CA) constructed in the lambda DASH vector was screened

at low stringency (Sambrook et al.,1989) with the MOP020 (511 bp) probe. The positive clones were purified to homogeneity and analysed by Southern blotting. The restriction map of the locus was determined and a relevant XbaI fragment of 4,400 bp was subcloned in pBluescript SK+ (Stratagene). Sequencing was performed on both strands after subcloning in M13mp derivatives, using fluorescent primers and an automated DNA sequencer (Applied Biosystem 370A). Sequence handling and data analysis was carried out using the DNASIS/PROSIS software (Hitachi), and the GCG software package (Genetics Computer Group, Wisconsin).

Expression in cell lines

The entire coding region was amplified by PCR as a 1056 bp fragment, using primers including respectively the *BamHI* and *XbaI* recognition sequences, and cloned after restriction in the corresponding sites of the eukaryotic expression vector pcDNA3 (Invitrogen, San Diego, CA). The resulting construct was verified by sequencing, and transfected in CHO-K1 cells as described (Perret et al., 1990). Two days after transfection, selection for stably transfected cell lines was initiated by the addition of 400 µg/ml G418 (Gibco), and resistant clones were isolated at day 10. CHO-K1 cells were cultured using Ham's F12 medium, as previously described (Perret et al., 1990; Desarnaud et al., 1994). The expression of the ChemR13 receptor in the various cell clones was evaluated by measuring the specific transcript level by Northern blotting, on total RNA prepared from the cells (see below).

Binding Assays

Stably transfected CHO-K1 cells expressing the ChemR13 receptor were grown to confluence and detached from culture dishes by incubation in phosphate-buffered saline (PBS) supplemented with 1 mM EDTA. Cells were collected by low speed centrifugation and counted in a Neubaeur cell. Binding assays were performed in polyethylene minisorp tubes (Nunc) in a final volume of 200 µl PBS containing 0.2 % bovine serum albumin (BSA) and 10^6 cells, in presence of [^{125}I]-MIP-1α. Non specific binding was determined by addition of 10 nM unlabelled MIP-1α. The concentration of labelled ligand was 0.4 nM (around 100 000 cpm per tube). The incubation was carried out for 2 hours at 4 °C, and was stopped by the rapid addition of 4 ml ice-cold buffer, and immediate collection of cells by vacuum filtration through GF/B glass fiber filters (Whatmann) pre-soaked in 0.5 % polyethyleneinimine (Sigma). Filters were washed three times with 4 ml ice-cold buffer and counted in a gamma counter.

Biological activity

The CHO-K1 cell lines stably transfected with the pcDNA3/ChemR13 construct or wild type CHO-K1 cells (used as controls) were plated onto the membrane of Transwell cell capsules (Molecular Devices), at a density of 2.5 10^5 cells/well in Ham's F12 medium. The next day, the capsules were transferred in a microphysiometer (Cytosensor, Molecular Devices), and the cells were allowed to equilibrate for approximately two hours by perifusion of 1 mM phosphate-buffered (pH 7.4) RPMI-1640 medium containing 0.2 % BSA. Cells were then exposed to various chemokines diluted in the same medium, for a 2 min duration. Acidification rates were measured at one minute intervals.

Northern blotting

Total RNA was isolated from transfected CHO-K1 cell lines, from a panel of human cell lines of haematopoietic origin and from a panel of dog tissues, using the RNeasy kit (Qiagen). RNA samples (10 µg per lane) were denatured in presence of glyoxal (Mc Master & Carmichael, 1977), fractionated on a 1 % agarose gel in a 10 mM phosphate buffer (pH 7.0), and transferred to nylon membranes (Pall Biodyne A, Glen Cove, NY) as described (Thomas, 1980). After baking, the blots were prehybridized for 4h at 42° C in a solution consisting of 50 % formamide, 5x Denhardt solution (1x Denhardt: 0.02 % Ficoll, 0.02 % polyvinylpyrrolidone, 0.02 % BSA), 5x SSPE (1x SSPE: 0.18 M NaCl, 10 mM Na phosphate, 1 mM EDTA pH 8.3), 0.3 % Sodium Dodecyl Sulphate (SDS), 250 µg per ml denaturated DNA from herring testes. DNA probes were (α^{32}P)-labelled by random priming (Feinberg & Vogelstein, 1983). Hybridizations were carried out for 12h at 42° C in the same solution containing 10 % (wt/vol) dextran sulphate and the heat denaturated probe. Filters were washed up to 0.1x SSC (1x SSC: 150 mM NaCl, 15 mM Na Citrate pH 7.0), 0.1 % SDS at 60° C and autoradiographed at - 70° C using Amersham ß-max films.

2. RESULTS AND DISCUSSION

Cloning and structural analysis

The sequence homology characterizing genes encoding G protein-coupled receptors has allowed the cloning by low stringency polymerase chain reaction (PCR) of new members of this gene family (Libert et al., 1989; Parmentier et al., 1989). One of the clones amplified from mouse genomic DNA, named MOP020 presented strong similarities with characterized

chemokine receptors, sharing 80 % identity with the MCP-1 receptor (CC-CKR2) (Charo et al., 1994), 65 % identity with the MIP-1α/RANTES receptor (CC-CKR1) (Neote et al., 1993), and 51 % identity with IL-8 receptors (Holmes et al., 1991; Murphy & Tiffany, 1991). The clone was used as a probe to screen a human genomic library. A total of 16 lambda phage clones were isolated. It was inferred from the restriction pattern of each clone and from partial sequence data that all clones were belonging to a single contig (see below) in which two different coding sequences were included. One of the coding sequences was identical to the reported cDNA encoding the CC-CKR2 receptor (Charo et al., 1994; Yamagani et al., 1994). A 4.400 pb *Xba*I fragment of a representative clone containing the second region of hybridization was subcloned in pBluescript SK+. Sequencing revealed a novel gene, tentatively named ChemR13, sharing 84 % identity with the MOP020 probe, suggesting that MOP020 is the mouse ortholog of ChemR13. MOP020 does not correspond to any of the three mouse chemokine receptor genes cloned recently (Gao & Murphy, 1995), demonstrating the existence of a fourth murine chemokine receptor.

The sequence of ChemR13 revealed a single open reading frame of 352 codons (fig. 1) encoding a protein of 40,600 Da. The sequence surrounding the proposed initiation codon is in agreement with the consensus as described by Kozak (Kozak, 1989), since the nucleotide in -3 is a purine. The hydropathy profile of the deduced amino acid sequence is consistent with the existence of 7 transmembrane segments. Alignment of the ChemR13 amino acid sequence with that of other functionally characterized human CC-chemokine receptors is represented in figure 1. The highest similarity is found with the CC-CKR2 receptor (Charo et al., 1994) that shares

75.8 % identical residues. There is also 56.3 % identity with the CC-CKR1 receptor (Neote et al., 1993), 58.4 % with the CC-CKR3 (Combadiere et al., 1993), and 49.1% with the CC-CKR4 (Power et al., 1995). ChemR13 represents therefore a new member of the CC-chemokine receptor group (Murphy, 1994). Like the related CC-CKR1 and IL-8 receptors (Holmes et al., 1991; Murphy & Tiffany, 1991; Neote et al., 1993; Gao et al., 1993) the coding region of ChemR13 appears as intronless. From our partial sequencing data, the CC-CKR2 gene is also devoid of intron in the first two thirds of its coding sequence.

Sequence similarities within the chemokine receptor family are higher in the transmembrane-spanning domains, and in intracellular loops. As an example, the identity score between ChemR13 and CC-CKR2 goes up to 92% when considering the transmembrane segments only. Lower similarities are found in the N-terminal extracellular domain, and in the extracellular loops. The N-terminal domain of the IL-8 and CC-CKR2 receptors has been shown to be essential for interaction with the ligand (Hébert et al., 1993; Gong & Clark-Lewis, 1995). The variability of this region among CC-chemokine receptors presumably contributes to the specificity towards the various ligands of the family.

A single potential site for N-linked glycosylation was identified in the third extracellular loop of ChemR13 (figure 1). No glycosylation site was found in the N-terminal domain of the receptor, where most G protein-coupled receptors are glycosylated. The other chemokine receptors CC-CKR1 and CC-CKR2 present such an N-linked glycosylation site in their N-terminal domain (Neote et al., 1993 ; Charo et al., 1994). By contrast, the CC-CKR3 receptor (Combadiere et al., 1995) does not display glycosylation sites neither in

the N-terminus, nor in extracellular loops. The ChemR13 receptor has four cysteines in its extracellular segments, and all four are conserved in the other CC- and CXC-chemokine receptors (figure 2). The cysteines located in the first and second extracellular loops are present in most G protein-coupled receptors, and are believed to form a disulphide bridge stabilizing the receptor structure (Strader et al., 1994). The two other cysteines, in the N-terminal segment, and in the third extracellular loop could similarly form a stabilizing bridge specific to the chemokine receptor family (Hébert et al., 1993). The intracellular domains of ChemR13 do not include potential sites for phosphorylation by protein kinase C (PKC) or protein kinase A. PKC sites, involved in heterologous desensitization are frequent in the third intracellular loop and C-terminus of G protein-coupled receptors. Such sites, present in CC-CKR3 and CC-CKR2 are represented in figure 1. CC-CKR1 is also devoid of PKC sites. In contrast, all CC-chemokine receptors, are rich in serine and threonine residues in the C-terminal domain. These residues represent potential phosphorylation sites by the family of G protein-coupled receptor kinases, and are probably involved in homologous desensitization (Strader et al., 1994). Five of these S/T residues are perfectly aligned in all five receptors (figure 2).

Physical linkage of the ChemR13 and CC-CKR2 genes

As stated above, the 16 clones isolated with the MOP020 probe corresponded to a single contig containing the ChemR13 and CC-CKR2 genes. The organisation of this contig was investigated in order to characterize the physical linkage of the two receptor genes in the human genome. A combination of restriction mapping, Southern blotting,

fragment subcloning and partial sequencing allowed to determine the respective borders and overlaps of all clones. Out of the 16 clones, 9 turned out to be characterized by a specific restriction map, and their organization is depicted in figure 3. Four of these clones (#11, 18, 21, 22) contained the CC-CKR2 gene alone, four clones (# 7, 13, 15, 16) contained the ChemR13 gene alone and one clone (#9) contains part of both coding sequences. The CC-CKR2 and ChemR13 genes are organized in tandem, ChemR13 being located downstream of CC-CKR2. The distance separating CC-CKR2 and ChemR13 open reading frames is 17.5 kb. The chromosomal localization of the tandem is presently unknown. Other chemokine receptors have however been located in the human genome: the CC-CKR1 gene was localized by fluorescence in situ hybridization to the p21 region of human chromosome 3 (Gao et al., 1993). The two IL-8 receptor genes, and their pseudogene have been shown to be clustered on the human 2q34-q35 region (Ahuja et al., 1992). Future studies will demonstrate if CC-chemokine receptor genes do form large clusters in the genome, as do the genes encoding their ligands (Baggiolini et al., 1994).

Functional expression and pharmacology of the ChemR13 receptor

Stable CHO-K1 cell lines expressing the ChemR13 receptor were established and were screened on the basis of the level of ChemR13 transcripts as determined by Northern blotting. Three clones were selected and tested for biological responses in a microphysiometer, using various CC- and CXC-chemokines as potential agonists. Wild type CHO-K1 cells were used as control to ensure that the observed responses were specific for the transfected receptor, and did not result from the activation of endogenous receptors. The

microphysiometer allows the real time detection of receptor activation, by measuring the modifications of cell metabolism resulting from the stimulation of intracellular cascades (Owicki & Parce, 1992). Several studies have already demonstrated the potential of microphysiometry in the field of chemokine receptors. Modifications of metabolic activity in human monocytes, in response CC-chemokines, were monitored using this system (Vaddi & Newton, 1994). Similarly, changes in the acidification rate of THP-1 cells (a human monocytic cell line) in response to MCP-1 and MCP-3 have been measured (Pleass et al., 1995). The estimation of the EC_{50} for both proteins, using this procedure, was in agreement with the values obtained by monitoring the intracellular calcium in other studies (Charo et al., 1994; Franci et al., 1995).

Ligands belonging to the CC- and CXC-chemokine classes were tested on the ChemR13 transfected CHO-K1 cells. Whereas MIP-1α, MIP-1ß and RANTES were found to be potent activators of the new receptor (figure 4), the CC-chemokines MCP-1, MCP-2 and MCP-3, and the CXC-chemokines GROα and IL-8 had no effect on the metabolic activity, even at the highest concentrations tested (30 nM). The biological activity of one of the chemokines inducing no response on ChemR13 (IL-8) could be demonstrated on a CHO-K1 cell line transfected with the IL-8A interleukin receptor (Mollereau et al., 1993) : IL-8 produced a 160 % increase in metabolic activity as determined using the microphysiometer. The biological activity of the MCP-2 and MCP-3 preparations as provided by J. Van Damme have been widely documented (Alam et al., 1994; Sozzani et al., 1994). MIP-1α, MIP-1ß and RANTES were tested on the wild type CHO-K1 cells, at a 30 nM-concentration, and none of them induced a metabolic response. On the ChemR13 transfected CHO-K1 cell line, all three active ligands

(MIP-1α, MIP-1ß and RANTES) caused a rapid increase in acidification rate, reaching a maximum by the second or third minute after perfusion of the ligand. The acidification rate returned to basal level within 10 minutes. The timing of the cellular response is similar to that observed for chemokines on their natural receptors in human monocytes (Vaddi & Newton, 1994). When agonists were applied repeatedly to the same cells, the response was strongly reduced as compared to the first stimulation, suggesting the desensitization of the receptor. All measurements were therefore obtained on the first stimulation of each capsule.

The concentration-effect relation was evaluated for the three active ligands in the 0.3 to 30 nM range (figure 3B and C). The rank order of potency was MIP-1α > MIP-1ß = RANTES. At 30 nM concentrations, the effect of MIP-1α appeared to saturate (at 156 % of baseline level) while MIP-1ß and RANTES were still in the ascending phase. Higher concentrations of chemokines could however not be used. The EC50 was estimated around 3 nM for MIP-1α. The concentrations necessary for obtaining a biological response as determined by using the microphysiometer are in the same range as those measured by intracellular calcium mobilization for the CC-CKR1 (Neote et al., 1993), the CC-CKR2A and B (Charo et al., 1994), and the CC-CKR3 (Combadiere et al., 1995) receptors. The ligand specificity of ChemR13 is similar to that reported for CC-CKR3 (Combadiere et al., 1995). CC-CKR3 was described as the first cloned receptor responding to MIP-1ß. However, MIP-1ß at 10 nM elicits a significant effect on the ChemR13, while the same concentration is without effect on the CC-CKR3 transfected cells (Combadiere et al., 1995). These data suggest that ChemR13 could be a physiological receptor for MIP-1ß.

Binding experiments using [^{125}I]-human MIP-1α as ligand did not allow to demonstrate specific binding to ChemR13 expressing CHO-K1 cells, using as much as 0.4 nM radioligand and 1 million transfected cells per tube. Failure to obtain binding data could be attributed to a relatively low affinity of the receptor for MIP-1α and possibly to an inadequate receptor expression level in our CHO cell line.

Northern blotting analysis

Northern blotting performed on a panel of dog tissues did not allow to detect transcripts for ChemR13. Given the role of the chemokine receptor family in mediating chemoattraction and activation of various classes of cells involved in inflammatory and immune responses, the probe was also used to detect specific transcripts in a panel of human cell lines of haematopoietic origin (figure 5). The panel included lymphoblastic (Raji) and T lymphoblastic (Jurkat) cell lines, promyeloblastic (KG-1A) and promyelocytic (HL-60) cell lines, a monocytic (THP-1) cell line, an erythroleukemia (HEL 92.1.7) cell line, a megakaryoblastic (MEG-01) cell line, and a myelogenous leukaemia (K-562) cell line. Human peripheral blood mononuclear cells (PBMC), including mature monocytes and lymphocytes, were also tested. ChemR13 transcripts (4.4 kb) could be detected only in the KG-1A promyeloblastic cell line, but were not found in the promyelocytic cell line HL-60, in PBMC, or in any of the other cell lines tested. These results suggest that the ChemR13 receptor could be expressed in precursors of the granulocytic lineage. CC-chemokines have been reported to stimulate mature granulocytes (McColl et al., 1993; Rot et al., 1992; Kuna et al., 1992; Alam et al., 1992). However, recent data have also demonstrated a role of CC- and CXC-

chemokines in the regulation of mousse and human myeloid progenitor cell proliferation (Broxmeyer et al., 1990; Broxmeyer et al., 1993). The expression of the ChemR13 receptor in normal precursor cells of the granulocyte lineage will nevertheless have to be confirmed.

REFERENCES

Ahuja, S.K., Oczelik, T., Milatovich, A., Francke, U., & Murphy, P.M. (1992) *Nature Genetics* 2, 31-36.

Alam, R., Forsythe, P., Stafford, S., Heinrich, J., Bravo, R., Proost, P., & VanDamme, J. (1994) *J. Immunol.* 153, 3155-3159.

Alam, R., Forsythe, P.A., Stafford, S., Lett-Brown, M.A., & Grant, J.A. (1992) *J. Exp. Med.* 176, 781-786.

Baggiolini, M., Dewald, B., & Moser, B. (1994) *Advances in immunology*, Academic press. Ed. Dixon, F.J. 55, 97-179.

Birkenbach, M., Josefsen, K., Yalamanchili, R., Lenoir, G., & Kieff, E. (1993) *J. Virol.* 67, 2209-2220.

Broxmeyer, H.E., Sherry, B., Lu, L., Cooper, S., Oh, K., Tekamp-Olson, P., Kwon, B.S., & Cerami, A. (1990) *Blood* 76, 1110-1116.

Broxmeyer, H.E., Sherry, B., Cooper, S., Lu, L., Maze, R., Beckmann, M.P., Cerami, A., & Ralph, P. (1993) *J. Immunol.* 150, 3448-3458.

Charo, I.F., Myers, S.J., Herman, A., Franci, C., Connolly, A.J., & Coughlin, S.R. (1994) *Proc. Natl. Acad. Sci.* 91, 2752-2756.

Cocci, F., DeVico, A.L., Garzino-Demo, A., Arya, S.K., Gallo, R.C., & Lusso, P. (1995) *Science* 270, 1811-1815.

Combadiere, C., Ahuja, S.K., & Murphy, P.M. (1995) *J. Biol. Chem.* 270, 16491-16494.

Desarnaud, F., Labbe, O., Eggerickx, D., Vassart, G., & Parmentier, M. (1994) *Biochem. J.* 299, 367-373.

Devereux, J., Haeberli, P., & Smithies, O. (1984) *Nucleic Acids Res.* 12, 387-395.

Dobner, T., Wolf, I., Emrich, T., & Lipp, M. (1992) *Eur. J. Immunol.* 22, 2795-2799.

Feinberg, A. P., & Vogelstein, B. (1983) *Anal. Biochem.* 132, 6-13.

Franci, C., Wong, L.M., Van Damme, J., Proost, P., & Charo, I.F. (1995) *J. Immunol.* 154, 6511-6517.

Gao, J.L., & Muphy, P.M. (1995) *J. Biol. Chem.* 270, 17494-17501.

Gao, J.L., Kuhns, D.B., Tiffany, H.L., McDermott, D., Li, X., Francke, U., & Murphy, P.M. (1993) *J. Exp. Med.* 177, 1421-1427.

Gong, J.H., & Clark-Lewis, I. (1995) *J. Exp. Med.* 181, 631-640.

Hébert, C.A., Chuntharapai, A., Smith, M., Colby, T., Kim, J., & Horuk, R. (1993) *J. Biol. Chem.* 268, 18549-18533.

Holmes, W.E., Lee, J., Kuang, V.J., Rice, G.C., & Wood, W.I. (1991) *Science* 253,1278-1283.

Jazin, E.E., Yoo, H., Blomqvist, A.G., Yee, F., Weng, G., Walker, M.W., Salon, J., Larhammar, D., & Washlestedt, C. (1993) *Regul. Peptides* 47, 247-258.

Kozak, M. (1989) *J. Cell. Biol.* 108, 229-241.

Kuna, P., Reddigari, S.R., Schall, T.J., Rucinski, D., Viksman, M.Y., & Kaplan, A.P. (1992) *J. Immunol.* 149, 636-642.

Libert, F., Parmentier, M., Lefort, A., Dinsart, C., Van Sande, J., Maenhaut, C., Simons, M. J., Dumont, J. E., & Vassart, G. (1989) *Science* 244, 569-572.

Matsuoka, I., Mori, T., Aoki, J., Sato, T., & Kurihara, K. (1993) *Biochem. Biophys. Res. Commun.* 194, 504-511.

Mc Master, G. K., & Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. USA* 74, 4835-4838.

McColl, S.R., Hachicha, M., Levasseur, S., Neote, K., & Schall, T.J. (1993) *J. Immunol.* 150, 4550-4560.

Mollereau, C., Muscatelli, F., Mattei, M.G., Vassart, G., & Parmentier, M. (1993) *Genomics* 16, 248-251.

Murphy, P.M. (1994) *Annu. Rev. Immunol.* 12, 593-633.

Murphy, P.M., & Tiffany, H.L. (1991) *Science* 253, 1280-1283.

Neote, K., DiGregorio, D., Mak, J.Y., Horuk, R., & Schall, T.J. (1993) *Cell* 72, 415-425.

Oppenheim, J.J., Zachariae, C.O., Mukaida, N., & Matsushima, K. (1991) *Ann. Rev. Immunol.* 9, 617-648.

Owicki, J.C., & Parce, J.W. (1992) *Biosensors Bioelectronics* 7, 255-272.

Parmentier, M., Libert, F., Maenhaut, C., Lefort, A., Gerard, C., Perret, J., Van Sande, J., Dumont, J. E., & Vassart, G. (1989) *Science* 246, 1620-1622.

Perret, J., Ludgate, M., Libert, F., Gérard, C., Dumont, J. E., Vassart, G., & Parmentier, M. (1990) *Biochem. Biophys. Res. Commun.* 17, 1044-1050.

Pleass, R.D., Blythe, T.C., Moore, U.M., Roach, A.G., & Williams, R.J. (1995) *Fourth International Chemokine Symposium*, Bath (UK), 27-30 June, P47.

Power, C.A., Meyer, A., Nemeth, K., Bacon, K.B., Hoogewerf, A.J., Proudfoot, A.E.I., & Wells T.N.C. (1995) *J. Biol. Chem.* 270, 19495-19500.

Rot, A., Krieger, M., Brunner, T., Bischoff, S.C., Schall, T.J., & Dahinden, C.A. (1992) *J. Exp. Med.* 176, 1489-1495.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Sozzani, S., Zhou, D., Locati, M., Rieppi, M., Proost, P., Magazin, M., Vita, N., VanDamme, J., & Mantovani, A. (1994) *J. Immunol.* 152, 3615-3622.

Strader, C.D., Fong, T.M., Tota, M.R., & Underwood, D. (1994) *Annu. Rev. Biochem.* Ed. Dixon, R.A.F. 63, 101-132.

Thomas, P. S. (1980). *Proc. Natl. Acad. Sci. USA* 77, 5201-5205.

Vaddi, K., & Newton, R.C. (1994) *The FASEB Journal* 8, A502.

Yamagami, S., Tokuda, Y., Ishii, K., Tanaka, H., & Endo, N. (1994) *Biochem. Biophys. Res. Commun.* 202, 1156-1162.

CLAIMS.

1. CC-chemokine receptor which is stimulated by the MIP-1ß chemokine at a concentration ≤ 10 nM.

2. Receptor according to the claim 1, which is stimulated by the MIP-1α and RANTES chemokines.

3. Receptor according to the claim 1 or 2, which is not stimulated by the MCP-1, MCP-2, MCP-3, IL-8 and GROα chemokines.

4. Receptor according to any of the preceding claims, being a human receptor.

5. Receptor according to any of the preceding claims, which has an amino acid sequence having more than 70% homology with the amino acid sequence shown in Figure 1.

6. Receptor according to any of the preceding claims, which has at least the amino acid sequence shown in Figure 1 or a portion thereof.

7. Nucleic acid molecule encoding the receptor according to any of the preceding claims.

8. Nucleic acid molecule according to claim 7, wherein the nucleic acid molecule is DNA or RNA molecule.

9. DNA molecule according to claim 8, which is a cDNA molecule or a genomic DNA molecule.

10. Nucleic acid molecule according to any of the claims 7 to 9, having more than 70% homology to the DNA sequence shown in Figure 1.

11. DNA molecule according to claim 10, which has at least the DNA sequence as shown in figure 1 or a portion thereof.

12. Vector comprising the nucleic acid molecule according to any of the claims 9 to 11.

13. Vector of claim 12, adapted for expression in a cell, which comprises the regulatory elements necessary for

expression of the nucleic acid molecule in said cell operatively linked to the nucleic acid molecule according to any of the claims 7 to 11 as to permit expression thereof.

14. Vector of claim 13, wherein the cell is chosen among the group consisting of bacterial cells, yeast cells, insect cells or mammalian cells.

15. Vector according to any of the claims 12 to 14, wherein the vector is a plasmid or a virus, preferably a baculovirus, an adenovirus or a semliki forest virus.

16. Vector of claim 15, wherein the plasmid is the pcDNA3 plasmid.

17. Cell comprising the vector according to any of the claims 12 to 16.

18. Cell according to claim 17, characterized in that it is transformed also by another vector encoding a protein enhancing the functional response in said cell, preferably said protein being the Gα15 or the Gα16 protein.

19. Cell of claim 17 or 18, wherein the cell is a mammalian cell, preferably non neuronal in origin.

20. Cell of claim 19, wherein the cell is chosen among the group consisting of CHO-K1, HEK293, BHK21, COS-7 cells.

21. Nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with an unique sequence included within the nucleic acid molecule according to any of the claims 7 to 11.

22. Nucleic acid probe of claim 21, wherein the nucleic acid is DNA or RNA.

23. Antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule of claim 8, so as to prevent translation of the mRNA molecule.

24. Antisense oligonucleotide having a sequence capable of specifically hybridizing to the DNA molecule of claim 9.

25. Antisense oligonucleotide according to claim 23 or 24, comprising chemical analogs of nucleotides.

26. Ligand capable of binding to a receptor according to any of the claims 1 to 6.

27. Anti-ligand capable of competitively inhibiting the binding of the ligand according to claim 26 to the receptor according to any of the claims 1 to 6.

28. Ligand according to claim 26, which is an antibody.

29. Anti-ligand according to the claim 27, which is an antibody.

30. Antibody according to the claim 28 or 29, which is a monoclonal antibody.

31. Monoclonal antibody according to claim 30, directed to an epitope of the receptor according to any of the claims 1 to 6, present on the surface of a cell expressing said receptor.

32. Pharmaceutical composition comprising an amount of the oligonucleotide according to claim 23, effective to decrease activity of the receptor according to any of the claims 1 to 6 by passing through a cell membrane and binding specifically with mRNA encoding said receptor in the cell so as to prevent its translation, and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

33. Pharmaceutical composition of claim 32, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

34. Pharmaceutical composition of claim 33, wherein the substance which inactivates mRNA is a ribozyme.

35. Pharmaceutical composition according to any of the claims 32 to 34, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cell after binding to the structure.

36. Pharmaceutical composition of claim 35, wherein the structure of the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.

37. Pharmaceutical composition which comprises an effective amount of the anti-ligand of claim 27, effective to block binding of a ligand to the receptor according to any of the claims 1 to 6 and a pharmaceutically acceptable carrier.

38. Transgenic non human mammal expressing the nucleic acid molecule according to any of the claims 7 to 11.

39. Transgenic non human mammal comprising an homologous recombination knockout of the native receptor according to any of the claims 1 to 6.

40. Transgenic non human mammal whose genome comprises antisense nucleic acid complementary to the nucleic acid molecule according to any of the claims 7 to 11 so placed as to be transcripted into antisense mRNA which is complementary to the mRNA of claim 8 and which hybridizes to said mRNA thereby reducing its translation.

41. Transgenic non human mammal according to any of the claims 38 to 40, wherein the nucleic acid according to any of the claims 7 to 11 additionally comprises an inducible promoter.

42. Transgenic non human mammal according to any of the claims 38 to 41, wherein the nucleic acid according to claim 7 to 11 additionally comprises tissue specific regulatory elements.

43. Transgenic non human mammal according to any of the claims 38 to 42, which is a mouse.

44. Method for determining whether a ligand can specifically bind to a receptor according to any of the claims 1 to 6, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand under conditions permitting binding of ligand to such receptor and detecting the presence of any such ligand bound specifically to said receptor, thereby determining whether the ligand binds specifically to said receptor.

45. Method for determining whether a ligand can specifically bind to the receptor according to any of the claims 1 to 6, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the ligand with the membrane fraction under conditions permitting binding of the ligand to such receptor and detecting the presence of any ligand bound to said receptor, thereby determining whether the compound is capable of specifically binding to said receptor.

46. Method for determining whether a ligand is an agonist of the receptor according to any of the claims 1 to 6, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand under conditions permitting the activation of a functional receptor response from the cell

and detecting by means of a bio-assay, such as a second messenger response, an increase in the receptor activity, thereby determining whether the ligand is a receptor agonist.

47. Method for determining whether a ligand is an agonist of the receptor according to any of the claims 1 to 6, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a second messenger response, an increase in the receptor activity, thereby determining whether the ligand is a receptor agonist.

48. Method for determining whether a ligand is an antagonist of the receptor according to any of the claims 1 to 6, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a second messenger response, a decrease in the receptor activity, thereby determining whether the ligand is a receptor antagonist.

49. Method for determining whether a ligand is an antagonist of the receptor according to any of the claims 1 to 6, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand in the presence of a known receptor agonist, under conditions permitting the activation of a

functional receptor response and detecting by means of a bio-assay, such as a second messenger response, a decrease in the receptor activity, thereby determining whether the ligand is a receptor antagonist.

50. A method according to any of the claims 44 to 49, wherein the second messenger assay comprises measurement of calcium ions (Ca^{2+}), inositol phosphates (such as IP_3), diacylglycerol (DAG) or cAMP.

51. Method according to any of the preceding claims 44 to 50, wherein the cell is a mammalian cell, preferably non neuronal in origin, and chosen among the group consisting of CHO-K1, HEK293, BHK21 nad COS-7 cells.

52. Method according to any of the preceding claims 44 to 51, wherein the ligand is not previously known.

53. Ligand detected by the method according to any of the preceding claims 44 to 52.

54. Pharmaceutical composition which comprises the ligand according to claim 53 and a pharmaceutically acceptable carrier.

55. Method of screening drugs to identify drugs which specifically bind to the receptor according to any of the claims 1 to 6 on the surface of the cell, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with a plurality of drugs under conditions permitting binding of said drugs to the receptor, and determining those drugs which specifically bind to the transfected cell, thereby identifying drugs which specifically bind to the receptor.

56. Method of screening drugs to identify drugs which specifically bind to the receptor according to any of the claims 1 to 6 on the surface of the cell, which comprises preparing a cell extract from cells transfected with a vector

expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs and determining those drugs which bind to the transfected cell, thereby identifying drugs which specifically bind to said receptor.

57. Method of screening drugs to identify drugs which act as agonists of the receptor according to any of the claims 1 to 6, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with a plurality of drugs under conditions permitting the activation of a functional receptor response, and determining those drugs which activates such receptor using a bio-assay, such as a second messenger response, thereby identifying drugs which act as receptor agonists.

58. Method of screening drugs to identify drugs which act as agonists of the receptor according to any of the claims 1 to 6, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs under conditions permitting the activation of a functional receptor response, and determining those drugs which activate such receptor using a bio-assay, such as a second messenger response, thereby identifying drugs which act as receptor agonists.

59. Method of screening drugs to identify drugs which act as antagonists of the receptor according to any of the claims 1 to 6, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with a plurality of drugs in the presence of a known receptor agonist, under conditions

permitting the activation of a functional receptor response, and determining those drugs which inhibit the activation of the receptor using a bio-assay, such as a second messenger response, thereby identifying drugs which act as receptor antagonists.

60. Method of screening drugs to identify drugs which act as antagonists of the receptor according to any of the claims 1 to 6, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs in presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response, and determining those drugs which inhibit the activation of the receptor using a bio-assay, such as a second messenger response, thereby identifying drugs which act as receptor antagonists.

61. Method according to any of the claims 46 to 49 and 57 to 60, wherein the functional response detected by means of a bioassay is detected and measered by a microphysiometer.

62. Drug detected by any of the methods according to claims 55 to 61.

63. Pharmaceutical composition comprising a drug according to claim 62 and a pharmaceutically acceptable carrier.

64. Method of detecting the expression of the receptor according to any of the claims 1 to 6, by detecting the presence of mRNA coding said receptor, which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe according to claim 20 under hybridizing conditions, and detecting the presence

of mRNA hybridized to the probe, thereby detecting the expression of the receptor by the cell.

65. Method of detecting the presence of the receptor according to any of the claims 1 to 6 on the surface of a cell, which comprises contacting the cell with the anti-ligand of claim 27 under conditions permitting binding of the antibody to the receptor, and detecting the presence of the antibody bound to the cell, thereby detecting the presence of the receptor on the surface of the cell.

66. Method of determining the physiological effects of expressing varying levels of the receptor according to any of the claims 1 to 6, which comprises producing a transgenic non human mammal according to any of the claims 38 to 43 whose levels of receptor expression are varied by use of an inducible promoter which regulates the receptor regulation.

67. Method of determining the physiological effects of expressing varying levels of the receptor according to any of the claims 1 to 6, which comprises producing a panel of transgenic non human mammals according to any of the claims 38 to 43, each expressing a different amount of said receptor.

68. Method for identifying an antagonist of the receptor according to any of the claims 1 to 6 capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of the receptor, which comprises administering the antagonist to a transgenic non human mammal according to any of the claims 38 to 43 and determining whether the antagonist alleviates the physical and behavioural abnormalities displayed by the transgenic non human mammal as a result of receptor activity, thereby identifying the antagonist.

69. Antagonist identified by any of the methods of the claims 64 to 68.

70. Pharmaceutical composition comprising an antagonist according to claim 69 and a pharmaceutically acceptable carrier.

71. Method for identifying an agonist of the receptor according to any of the claims 1 to 6 capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by activation of said receptor, which comprises administering the agonist to a transgenic non human mammal according to any of the claims 38 to 43 and determining whether the antagonist alleviates the physical and behavioural abnormalities displayed by the transgenic non human mammal, the alleviation of the abnormalities indicating the identification of the agonist.

72. Agonist identified by the method of claim 71.

73. Pharmaceutical composition comprising an agonist according to claim 72 and a pharmaceutically acceptable carrier.

74. Method for diagnosing a predisposition to a disorder associated with the activity of a specific allele of the receptor according to any of the claims 1 to 6, which comprises :

a) obtaining nucleic acid molecules of subjects suffering from said disorder;

b) performing a restriction digest of said nucleic acid molecules with a panel of restriction enzymes;

c) electrophoretically separating the resulting nucleic acid fragments on a sized gel;

d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to said nucleic acid molecule and labelled with a detectable marker;

e) detecting labelled bands which have hybridized to the said nucleic acid molecule labelled with a detectable marker to create a unique band pattern specific to subjects suffering from said disorder;

f) preparing nucleic acid molecules obtained for diagnosis by step a-e; and

g) comparing the unique band pattern specific to the nucleic acid molecule of subjects suffering from the disorder from step e and the nucleic acid molecule obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

75. Method of preparing the purified receptor according to any of the claims 1 to 6, which comprises :

a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid molecules in the cell operatively linked to nucleic acid molecule encoding said receptor so as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;

b) inserting the vector of step a in a suitable host cell;

c) incubating the cell of step b under conditions allowing the expression of the receptor according to the invention;

d) recovering the receptor so obtained; and

e) purifying the receptor so recovered, thereby preparing an isolated receptor according to the invention.

76. Use of the pharmaceutical composition according to any of the claims 32 to 37, 54, 63, 70 and 73, for the preparation of a medicament in the treatment of a disease

chosen from the group consisting of inflammatory diseases, including rheumatoid arthritis, glomerulonephritis, asthma, idiopathic pulmonary fibrosis and psoriasis, viral infections including infections by Human Immunodeficiency Viruses 1 and 2 (HIV-1 and 2), cancer including leukaemia, atherosclerosis and/or auto-immune disorders.

ABSTRACT

CC-CHEMOKINES RECEPTOR AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR.

The present invention concerns a new CC-chemokine receptor which is stimulated by the MIP-1ß chemokine at a concentration ≤ 10 nM.

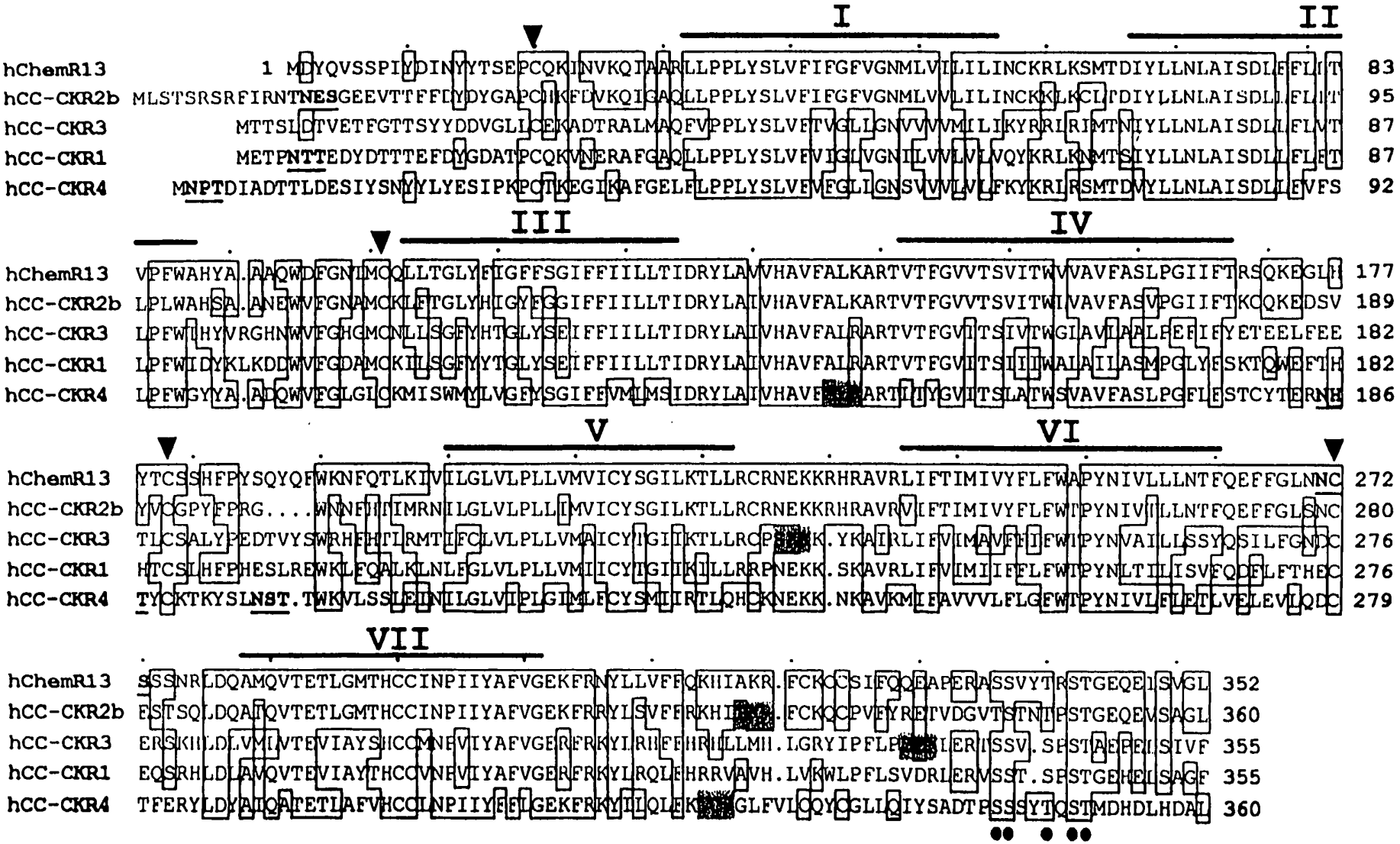
The present invention concerns also the nucleic acid molecule encoding said receptor, the vector comprising said nucleic acid molecule, cells transformed by said vector, antibodies directed against said receptor, nucleic acid probes directed against said nucleic acid molecule, pharmaceutical compositions comprising said products and non-human transgenic animals expressing the receptor according to the invention or the nucleic acid molecule encoding said receptor.

(Figure 5)

```
                                                                    1
       GAATTCCCCCAACAGAGCCAAGCTCTCCATCTAGTGGACAGGGAAGCTAGCAGCAAACC      59
   60  TTCCCTTCACTACAAAACTTCATTGCTTGGCCAAAAAGAGAGTTAATTCAATGTAGACAT     119
  120  CTATGTAGGCAATTAAAAAACCTATTGATGTATAAAACAGTTTGCATTCATGGAGGGCAAC     179
                                                    M  E  G  N        4

  180  TAAATACATTCTAGGACTTTATAAAAGATCACTTTTTATTTATGCACAGGGTGGAACAAG     239
    5   *  I  H  S  R  T  L  *  K  I  T  F  Y  L  C  T  G  W  N  K      24

  240  ATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTATTATACATCGGAGCCCTGC     299
   25   M  D  Y  Q  V  S  S  P  I  Y  D  I  N  Y  Y  T  S  E  P  C      44

  300  CAAAAAATCAATGTGAAGCAAATCGCAGCCCGCCTCCTGCCTCCGCTCTACTCACTGGTG     359
   45   Q  K  I  N  V  K  Q  I  A  A  R  L  L  P  P  L  Y  S  L  V      64

  360  TTCATCTTTGGTTTTGTGGGCAACATGCTGGTCATCCTCATCCTGATAAACTGCAAAAGG     419
   65   F  I  F  G  F  V  G  N  M  L  V  I  L  I  L  I  N  C  K  R      84

  420  CTGAAGAGCATGACTGACATCTACCTGCTCAACCTGGCCATCTCTGACCTGTTTTTCCTT     479
   85   L  K  S  M  T  D  I  Y  L  L  N  L  A  I  S  D  L  F  F  L     104

  480  CTTACTGTCCCCTTCTGGGCTCACTATGCTGCCGCCCAGTGGGACTTTGGAAATACAATG     539
  105   L  T  V  P  F  W  A  H  Y  A  A  A  Q  W  D  F  G  N  T  M     124

  540  TGTCAACTCTTGACAGGGCTCTATTTTATAGGCTTCTTCTCTGGAATCTTCTTCATCATC     599
  125   C  Q  L  L  T  G  L  Y  F  I  G  F  F  S  G  I  F  F  I  I     144

  600  CTCCTGACAATCGATAGGTACCTGGCTGTCGTCCATGCTGTGTTTGCTTTAAAAGCCAGG     659
  145   L  L  T  I  D  R  Y  L  A  V  V  H  A  V  F  A  L  K  A  R     164

  660  ACGGTCACCTTTGGGGTGGTGACAAGTGTGATCACTTGGGTGGTGGCTGTGTTTGCGTCT     719
  165   T  V  T  F  G  V  V  T  S  V  I  T  W  V  V  A  V  F  A  S     184

  720  CTCCCAGGAATCATCTTTACCAGATCTCAAAAAGAAGGTCTTCATTACACCTGCAGCTCT     779
  185   L  P  G  I  I  F  T  R  S  Q  K  E  G  L  H  Y  T  C  S  S     204

  780  CATTTTCCATACAGTCAGTATCAATTCTGGAAGAATTTCCAGACATTAAAGATAGTCATC     839
  205   H  F  P  Y  S  Q  Y  Q  F  W  K  N  F  Q  T  L  K  I  V  I     224

  840  TTGGGGCTGGTCCTGCCGCTGCTTGTCATGGTCATCTGCTACTCGGGAATCCTAAAAACT     899
  225   L  G  L  V  L  P  L  L  V  M  V  I  C  Y  S  G  I  L  K  T     244

  900  CTGCTTCGGTGTCGAAATGAGAAGAAGAGGCACAGGGCTGTGAGGCTTATCTTCACCATC     959
  245   L  L  R  C  R  N  E  K  K  R  H  R  A  V  R  L  I  F  T  I     264

  960  ATGATTGTTTATTTTCTCTTCTGGGCTCCCTACAACATTGTCCTTCTCCTGAACACCTTC    1019
  265   M  I  V  Y  F  L  F  W  A  P  Y  N  I  V  L  L  L  N  T  F     284

 1020  CAGGAATTCTTTGGCCTGAATAATTGCAGTAGCTCTAACAGGTTGGACCAAGCTATGCAG    1079
  285   Q  E  F  F  G  L  N  N  C  S  S  S  N  R  L  D  Q  A  M  Q     304

 1080  GTGACAGAGACTCTTGGGATGACGCACTGCTGCATCAACCCCATCATCTATGCCTTTGTC    1139
  305   V  T  E  T  L  G  M  T  H  C  C  I  N  P  I  I  Y  A  F  V     324

 1140  GGGGAGAAGTTCAGAAACTACCTCTTAGTCTTCTTCCAAAAGCACATTGCCAAACGCTTC    1199
  325   G  E  K  F  R  N  Y  L  L  V  F  F  Q  K  H  I  A  K  R  F     344

 1200  TGCAAATGCTGTTCTATTTTCCAGCAAGAGGCTCCCGAGCGAGCAAGCTCAGTTTACACC    1259
  345   C  K  C  C  S  I  F  Q  Q  E  A  P  E  R  A  S  S  V  Y  T     364

 1260  CGATCCACTGGGGAGCAGGAAATATCTGTGGGCTTGTGACACGGACTCAAGTGGGCTGGT    1319
  365   R  S  T  G  E  Q  E  I  S  V  G  L  *

 1320  GACCCAGTCAGAGTTGTGCACATGGCTTAGTTTTCATACACAGCCTGGGCTGGGGGTNGG    1379

 1380  TTGGNNGAGGTCTTTTTTAAAAGGAAGTTACTGTTATAGAGGGTCTAAGATTCATCCATT    1439

 1440  TATTTGGCATCTGTTTAAAGTAGATTAGATCCGAATTC
```

FIG.1

FIG.2


I
II
III
IV
V
VI
VII
hChemR13
hCC-CKR2b
hCC-CKR3
hCC-CKR1
hCC-CKR4

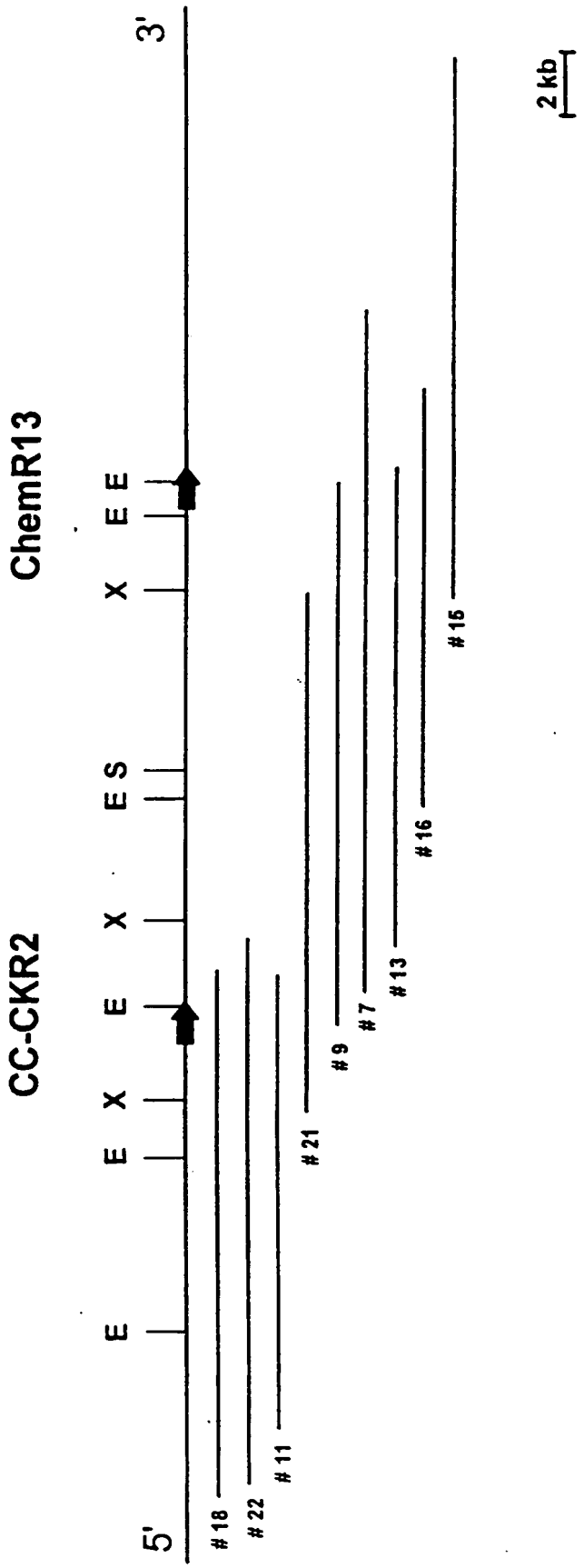


FIG.3

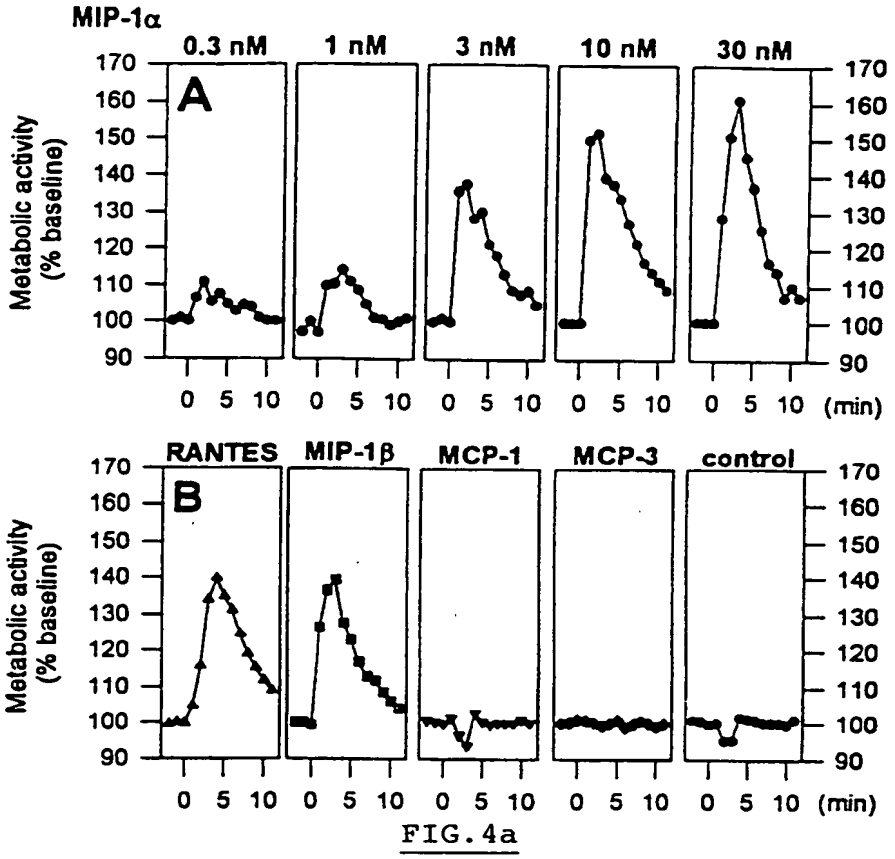

MIP-1α
0.3 nM
1 nM
3 nM
10 nM
30 nM
A
Metabolic activity (% baseline)
170
160
150
140
130
120
110
100
90
0 5 10
(min)
RANTES
MIP-1β
MCP-1
MCP-3
control
B

FIG.4a

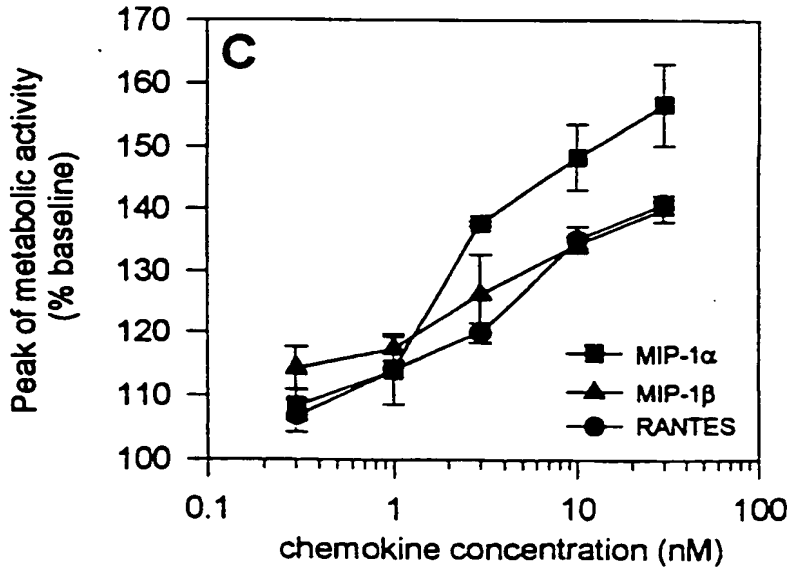

C
Peak of metabolic activity (% baseline)
170
160
150
140
130
120
110
100
0.1
1
10
100
chemokine concentration (nM)
MIP-1α
MIP-1β
RANTES

FIG.4b

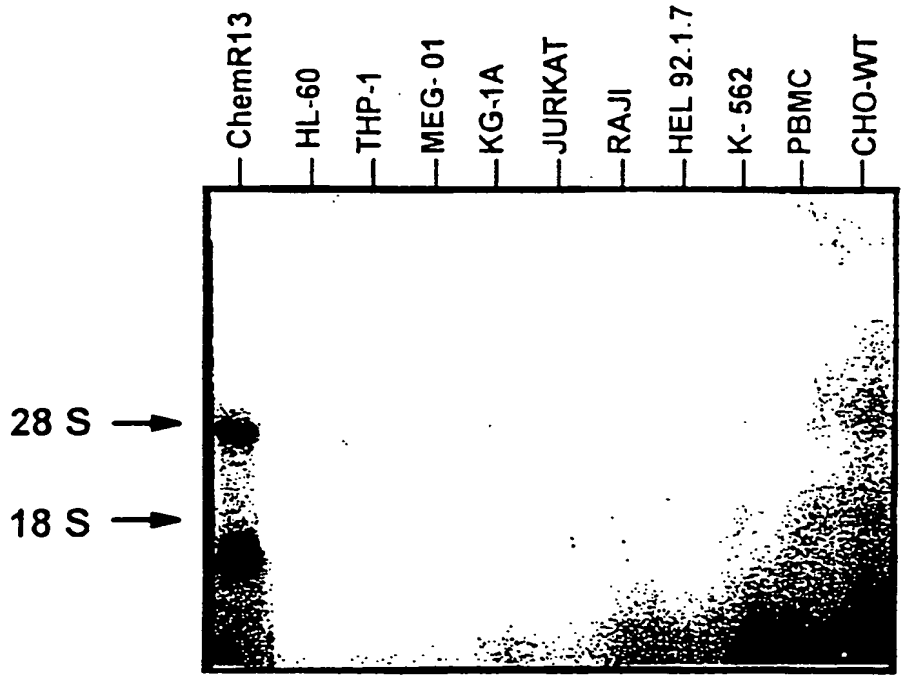

ChemR13
HL-60
THP-1
MEG-01
KG-1A
JURKAT
RAJI
HEL 92.1.7
K-562
PBMC
CHO-WT
28 S
18 S

FIG.5